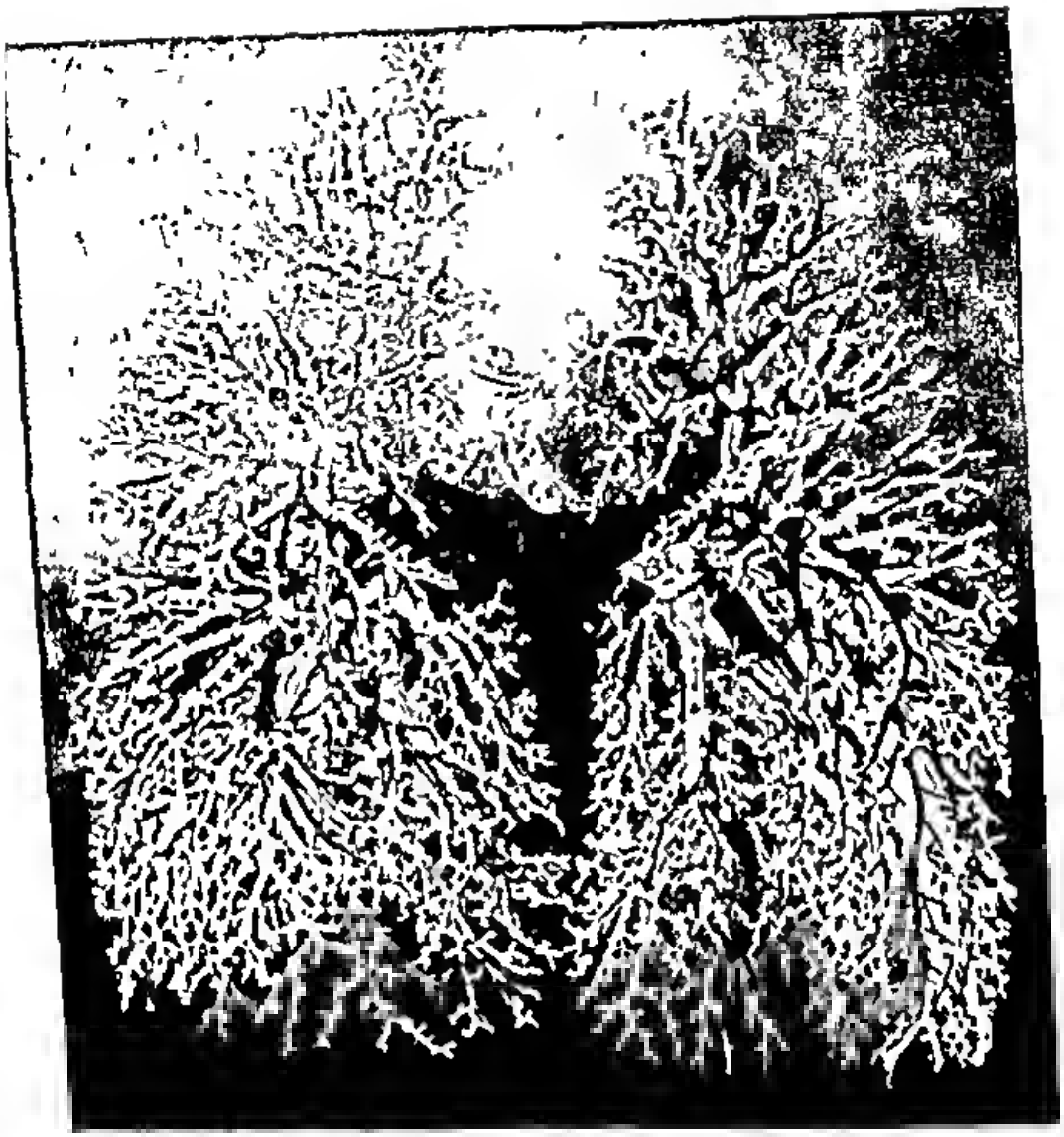
ANATOMICAL TECHNIQUES



A corrosion cast of an adult human bronchial tree, together with the pulmonary arteries × ½.

[*Frontispiece*

ANATOMICAL TECHNIQUES

BY

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B Sc, Ph D

Prosector to the Royal College of Surgeons of England

FOREWORD BY

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HISTORICAL INTRODUCTION

BY

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Foreword

I am very pleased to write a Foreword to this instructive and very practical book because I feel it is a book which should interest not only students but also surgeons who participate in the teaching of anatomy and surgery. It is far more fascinating and realistic to teach from specimens made from the human body than from illustrations or drawings.

I have watched Dr. Tompsett's work over many years especially during those years when I was President of the Royal College of Surgeons and was constantly in all departments of the College. I feel that the author of this book has followed the true Hunterian tradition and has brought forward ways and means of displaying anatomical detail in a way which is quite unique and deserves much credit.

In my opinion this is one of the really useful books for the student anatomist and surgeon alike. The College of Surgeons may well be proud of this work which has emanated from within its walls. It is clearly written, lavishly illustrated and should prove invaluable to all who are interested in the anatomy of the human body.

Cecil Wakeley

Preface

The majority of the specimens in anatomy museums have been prepared by medical students or lecturers, who do this work, partly because they find it satisfying, and partly because there is a pressing need for the specimens for teaching purposes. But they have to do it in their spare time, apart from the real business of their lives.

I have written this book primarily to help such part-time workers, since, as a professional prosector, I have been able for ten years to devote all my energy to the perfection of the art of dissection, and the development of various techniques.

Some people have been surprised that my qualifications do not include a medical degree. But as the latter involves specialisation in a narrow field, I am sure that my actual qualifications, *viz.*, a general science degree in Chemistry, Botany and Zoology, a Ph.D. in Zoology and a Diploma of Education (and also a year's preliminary training in Engineering, which has proved very useful on occasions) have formed a broader and generally sounder basis for my subsequent work than would have been provided by only a medical qualification.

A lack of detailed knowledge of human anatomy is no handicap to the production of accurate preparations; indeed in many cases it proves an actual advantage, in that the dissection cannot be modelled on preconceived ideas. The essential qualifications for this type of work are general aptitude and sustained interest. Although patience is also necessary, this is only a form of self-discipline, which I for one have found exceedingly difficult to acquire, being by temperament a very impatient person.

The techniques described in this volume are limited to those concerned with the preparation of museum specimens of human anatomy, of which I have had intimate practical experience. They can however be adapted for making preparations to illustrate comparative vertebrate anatomy. For work on invertebrates considerable modifications of technique are sometimes necessary.

D. H. Tompsett.

London, 1956

Acknowledgments

I acknowledge with thanks the help I have received from Professor G. W. Causey and members of the staff of the department of anatomy of the Royal College of Surgeons of England during the writing of this book. I am particularly indebted to two members. Professor R. J. Last has taken a close interest in my work for more than eight years, and his intimate knowledge of anatomy has always been at my service, to compensate for my deficiency in this subject. He has also read the draft of each chapter, and his critical judgment has been of the greatest assistance to me. I am also indebted to Mr. S. C. Bartlett, museum technician to the department of anatomy for over thirty years, for much valuable information concerning embalming and mounting techniques. Mr. Bartlett has made many contributions to the perfection of mounting techniques described in this book.

I wish to thank the editors of the journals concerned for permission to include material originally published under the following titles :

1. A new method for the preparation of bronchopulmonary casts *Thorax* (1952) **7**, 78
2. A method of making a hollow model of the cerebral ventricular system The *British Journal of Radiology* (1953) **26**, 122.
3. Casts of the cerebral ventricles. The *British Journal of Surgery* (1953) **40**, 525
4. A technique for preparing a cast in synthetic resin of the cavities and blood vessels of the heart. *Thorax* (1954) **9**, 123
5. A method of making a cast in synthetic resin of the bronchial arteries *Thorax* (1954) **9**, 229.
6. A method of making a transparent cast of the temporal bone The *Journal of Laryngology and Otology* (1954) **68**, 805
7. Differential staining and mounting of human brain slices *Medical and Biological Illustration* (1955) **5**, 29

I am grateful to the editor of *Thorax* for the loan of the blocks for Figures 32, 37, 38, 40, 41, 42, 43 and 44; and to the editor of the *British Journal of Surgery* for Figures 53, 54, 58, 59, 60 and 61; to the editor of *Medical and Biological Illustration* for Figures 80, 81, 82 and 83.

I am indebted to Bayer Products Ltd. for the colour frontispiece, and for the colour blocks for Figures 45, 76 and 79; to Professor Last and

J. and A. Churchill Ltd. for permission to reproduce Figure 26; to Mr. B. Brake for Figure 11; and to Allen and Hanbury Ltd. for Figures 8, 9 and 10.

My work on casting with synthetic resin has been greatly facilitated by the cooperation, over the last six years, of Scott Bader and Co. Ltd. I also wish to thank the Plastics Division of Imperial Chemical Industries Ltd. for technical advice concerning methods of using Tensol No. 3 cement, and Hardman and Holden for advice concerning Manoxol O.T.

Finally I would like to express my appreciation of the unfailing help and cooperation extended to me by the publishers, and particularly by their Managing Director, Mr. Charles Macmillan.

Historical Introduction

It is obviously impossible in this brief survey to do more than give passing reference to the more outstanding of those who did pioneer work in the field of anatomical investigation and paved the way for the establishment of the modern anatomical museum.

The history of the preservation and preparation of anatomical specimens is inevitably linked with the history of the study of anatomy itself. In the six hundred years from 400 B.C. to 200 A.D. knowledge of the internal structure of the human body had reached an advanced stage, thanks to the investigations of Hippocrates, Celsus and Galen and their pupils. After the decline of the Roman Empire, however, the study of anatomy and, indeed, of many other branches of learning ceased to make such progress and for over a thousand years scholars continued to rely on the writings of these early scientists, making little effort, apparently, either to confirm their findings or to make appreciable additions to our knowledge. The renaissance in the science of anatomy may be said to have begun, therefore, with Leonardo da Vinci (1452-1519) who seems to have been the first to make systematic topographical dissections and serial sections to illustrate the structure of the body. This " modern biologist in the guise of a mediaeval artist " (F. J. Cole) was the first to give a representation of the cerebral ventricles by the injection of a solidifying substance; and he prepared a wax cast of the heart from which he made a glass model in order to determine the flow of blood within the organ.

Foremost among those who at this period sought after truth and accuracy was Andreas Vesalius (1514-1564) who antagonised his contemporaries by pointing out the errors in Galen's work. Throughout his investigations he followed the principle of accepting no authority save that of his own eyes. His *De Humani Corporis Fabrica*, published in 1543, stands unique as a dissecting manual and textbook of anatomy both for its clarity of exposition and perfection in illustration. When lack of material threatened to limit his enquiries, he risked his life and his reputation among his colleagues by removing bodies from the gibbets of Montfauçon and from the graves at the Cemetery of the Innocents in Paris; the result was that he was able to prove that Galen had frequently based his observations on findings in mammals other than man. This same adventurous spirit moved Paracelsus (1490-1541) to burn publicly the works of Galen and Avicenna in Basle as a protest against the blind acceptance of the authenticity of antiquity.

Linked with this revival of learning was the awakening of interest in natural history and the founding of museums, but the object in view in the arrangement of the specimens therein seems in many cases to have been to create surprise rather than to afford instruction. A typical museum of this period was that of Robert Hubert, alias Forges, which contained "many Natural Rarities, collected with great industry, cost, and thirty years' travel in foreign countries." This collection was removed to the continent during the Civil War but, on its return to England in 1664, was "dayly to be seen at the place called the Musick House, at the Miter, near the west end of St. Paul's Church." A few years later, part of it was purchased by the Royal Society and the rest by Sir Hans Sloane who also acquired the collection of William Charleton. Similarly the two Tradescents, at the beginning of the seventeenth century, built up a museum considered to be the most extensive in Europe, but of little scientific value for want of proper arrangement. This collection was bought by Elias Ashmole in 1659 and formed the basis of the present Ashmolean Museum. On the continent, perhaps the most celebrated collections were those of Ole Worm, Levinus Vincent, Theodor Kerckring and Albertus Seba.

The mediaeval museum consisted for the most part of collections of non-perishable objects such as shells, bones and fossils; the only anatomical specimens that could be included were dried and varnished viscera bearing little resemblance to the actual structure. It will thus be realised what an incalculable advance was made in museum technique by Robert Boyle's experiment, carried out on William Croune's suggestion, of preserving parts of, or even whole, animals in spirit. Boyle's specimens of 'a linnet and a little snake, preserved already four months, entrails and all, without any change in colour, in some spirit of wine,' placed in the repository of the Royal Society at Gresham College in 1663, were still in existence a hundred and fifty years later. This long-sought procedure for defying putrefaction did not mean, however, that the style of the museum immediately changed, for the prohibitive cost of the spirit and the glass containers for such 'wet' specimens restricted the numbers included in most collections.

But the need for more lasting preparations was urgent and the possibilities of fixatives less expensive than spirit were re-explored. Boyle suggested using isinglass or saccarum Saturni for this purpose, but whether he actually made the experiment is uncertain. At first, the preparation of injected specimens was just a part of the experimental work of the research worker and the results were not necessarily intended to be preserved indefinitely. Hence the

'founder of microscopical anatomy,' is usually regarded as being the first to have experimented with mercury injections and in 1661 by this means he demonstrated the structure of the lungs. Anthony Nuck (1650-1692) was the first to make use of this injection medium for the lymphatic system. He was misled in his interpretation of his findings, however, owing to the extravasation of the injection mass, but his general description of these structures is one of the most complete prior to that of Paolo Mascagni (1752-1815) a hundred years later.

In the eighteenth century this technique was carried to an even higher degree of perfection by William Hunter (1718-1783), William Hewson (1739-1774), William Cumberland Cruikshank (1746-1800) and William Sheldon (1752-1806). In his *History of the Absorbent System*, published in 1784, Sheldon gives an excellent description of the methods of injecting and preparing the lacteal vessels and the instruments used for the purpose. In an unpublished paper, preserved at the Royal Society and dated 1784, Joshua Brookes (1761-1833) gives what may well have been the first instructions for the preservation of dissecting-room subjects, though Henry Cline states that Frank Nicholls (1699-1778) was the first to use specially prepared and preserved specimens in his demonstrations to medical students. John Hunter, typically, did not confine himself to any particular type of injection, but used whichever method appeared to him best to show what he required in the finished preparation. Many of his injected specimens can still be seen in the Museum of the Royal College of Surgeons.

The new techniques brought with them the necessity for devising instruments suitable for their operation. Regnier de Graaf's (1641-1673) injecting syringe was a forerunner of the modern design and Caspar Bartholin (1655-1738), James Drake (1667-1707) and Alexander Monro (1697-1767) improved on this to ensure a more continuous feed. Instruction for the preparation of specimens and a description of the requisite instruments is usually to be found in the literature presenting the researches of the individual workers. There are very few instances of books devoted to this subject alone. The *Cultter Anatomicus* of Michael Lyser, published in 1653, is noteworthy, however, as being the first general treatise on anatomical methods. His desiderata, from which the following are selected, are as requisite to-day as they were three hundred years ago: "Postquam corpus delectum est, instrumenta ad operationem necessaria erunt conquirenda . . . Scalpella ex optimo chalybe debent, quorum acies cum novaculis decertet . . . Locum in quo peragitur sectio luminosus sit, ut scilicet minutiae corporis partes, quae

visum saepe fugiunt, accuratius cernantur"—having selected your subject, obtain the instruments suitable for the task; the scalpel should be of the best steel and sharp as a razor, and the place where the work is to take place must be well lighted in order that the smallest details should not escape notice. Other works worthy of mention in this connection are Johannes Friedrich Cassebohm's *Methodus secandi et contemplandi corporis humani musculus*, published in 1740; Jean Joseph Sue's *L'Anthropotomie ou l'art d'injecter* of 1749, Thomas Pole's *Anatomical Instructor* of 1790; and Robert Hooper's *Anatomists' Vade-mecum*, first published in 1797.

Towards the end of the seventeenth century, therefore, a noticeable change appeared in the content and arrangement of museums and particular mention must be made here of Friedrich Ruysch's first and main collection, which contained over thirteen hundred anatomical preparations in spirit. It is unfortunate that, unlike those whose names are mentioned above, he kept his methods secret so that, as William Wagstaffe points out in his Preface to James Drake's *Anthropologia Nova*, "Dr. Ruysch has given us in his *Thesaurus* several excellent and curious drawings of the finest preparations in the World, but we had certainly been more obliged to him if he had communicated his Observations on the Manner of preparing them and form'd from thence a noble, a just and a demonstrative Rationale of the Uses of the Parts and Morbid Alterations of our Frame." It is said that the bodies injected by Ruysch preserved all the freshness of youth so it is possible that his method was similar to that used by Charles White (1728-1813) for the 'Manchester Mummy,' and by William Sheldon and William Hunter in 1775 for the embalmed female bodies that survived in the museum of the Royal College of Surgeons until it was bombed in 1941. Ruysch's museum was purchased by Peter the Great and in 1717 was removed to St. Petersburg so that, unlike so many fine collections of the period, his life's work remained intact. That of Joshua Brookes was estimated to have cost £30,000 for he sacrificed everything to its improvement; but he was forced to sacrifice it in the end and in 1830 it was sold piecemeal for a fraction of its value. The collections of John Heaviside and George Langstaff, disposed of in 1829 and 1842 respectively, had a similar fate. Even Sir Joseph Bank's specimens were dispersed, some going to John Hunter and the rest to the British Museum: so, in the majority of cases, these first-hand illustrations of discoveries and techniques have been lost to us.

During the last hundred years an important change has taken place in the style and purpose of the museum. There are now apparently, very few of those 'curious' individuals who amass and treasure objects that arouse their

particular interest and so the vast private collections have, for the most part, disappeared; but, in place, there has developed the public, the instructive and the specialised museum in which the ordered arrangement and perfection of preparation and presentation of the contents are a mirror of the progress in knowledge and technique. It is in this period that an important discovery was made, comparable in its effect to that of Robert Boyle three hundred years previously. In 1845, August Wilhelm von Hofmann, born in Giessen in 1818, accepted the invitation, made at the suggestion of the Prince Consort, to become first director of the new Royal College of Chemistry in London. In 1856 he was appointed chemist to the Royal Mint and it was in 1863 that he discovered the gas, formic aldehyde. The forty per cent solution of this gas, now known as formalin, was used for many purposes but it was not until about thirty years later that Ferdinand Julius Cohn (1828-1898), the botanist of Breslau, experimented with it as a preserving agent for plants. Zoologists and anatomists soon found a suitable formula for its use as a preservative and fixative and, as Dr. Ryfkogel has pointed out (*Medical Record*, 50, 192), it possesses most of the desirable qualities of other fixatives with few of their disadvantages, since it does not cause excessive shrinkage or hardening, if the correct proportions are used: furthermore, it is non-inflammable and of little cost.

This introduction would not be complete without some mention of John Hunter's famous collection which is housed at the Royal College of Surgeons. At the time of his death in 1793 it contained no less than 13,682 specimens, each of which was specially prepared by him to demonstrate the story of life in the normal and the abnormal state, laying a particular stress on the adaptation of structure to function. It thus provides not only a record of the founder's industry, interests and ingenuity but is one of the very few surviving examples of an eighteenth century museum. Enriched by the activities of conservators such as Richard Owen and William Henry Flower, it was the mecca of the medical historian as well as of the student of human and comparative anatomy, physiology and pathology. Considerably more than half of this fine collection was lost as a result of bombing in 1941 but sufficient of the original specimens are left to reflect John Hunter's ideas and ideals and to form the basis for restoration. And for those whose agreeable task it is to help in its reconstitution, as well as for all those engaged in the preparation of museum specimens, the following pages will afford a reliable and inspiring guide.

JESSIE DOBSON.

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The Preparation and Mounting of differentially Stained Brain Slices

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PART I

THE PREPARATION AND MOUNTING OF ANATOMICAL SPECIMENS OF HUMAN PARTS

Chapter 1

INTRODUCTION

IN the preparation of museum dissections, the general procedure differs in several respects from that followed by medical students in the dissecting room. Provided that he finds the various structures and observes their relationships, the medical student is satisfied. He works speedily, because dissection is always very time-consuming. A torn artery or a ragged muscle does not matter, as it will be removed later in the examination of deeper structures; and if the surface of his dissection gets a little dry and consequently discoloured, it does not disconcert him unduly. Elaborate cleaning of the structures dissected is discouraged as being a waste of time.

When a museum dissection is being prepared, the principal structures to be displayed must be decided before the dissection is begun, and the dissection must be undertaken with extreme caution, to avoid damage to any structure which may be left in the finished specimen. The most painstaking cleaning of each structure and in addition a specialised finishing technique, are necessary to give the dissection a satisfactory appearance when it is immersed in mounting fluid. Otherwise what appears to be a neat and clean dissection when it rests on the table is seen to be clothed in a mass of ragged ends of connective tissue when mounted.

It is reported that when Mr. Pearson, prosector to the Royal College of Surgeons of England from 1890-1914, was asked the secret of how he made his very beautiful dissections of human anatomy, some of which can still be seen in the College museum, he replied : "I begin where you leave off." But while there was doubtless a great deal of truth in this statement, which implied that the beauty of Mr. Pearson's dissections resulted from his great skill and patience in cleaning the structures, it did not tell the whole story. It did not, for instance, explain why, when he was finishing a dissection, he locked himself in his laboratory for periods of up to thirty hours. There can be little doubt that Mr. Pearson's wonderful dissections owe some of their beauty to a special finishing technique, the details of which he kept as a jealously guarded secret. It is the ambition of the present writer to reveal as completely as possible all the tricks of his trade.

The difficulty of producing really first class human dissections is greatly

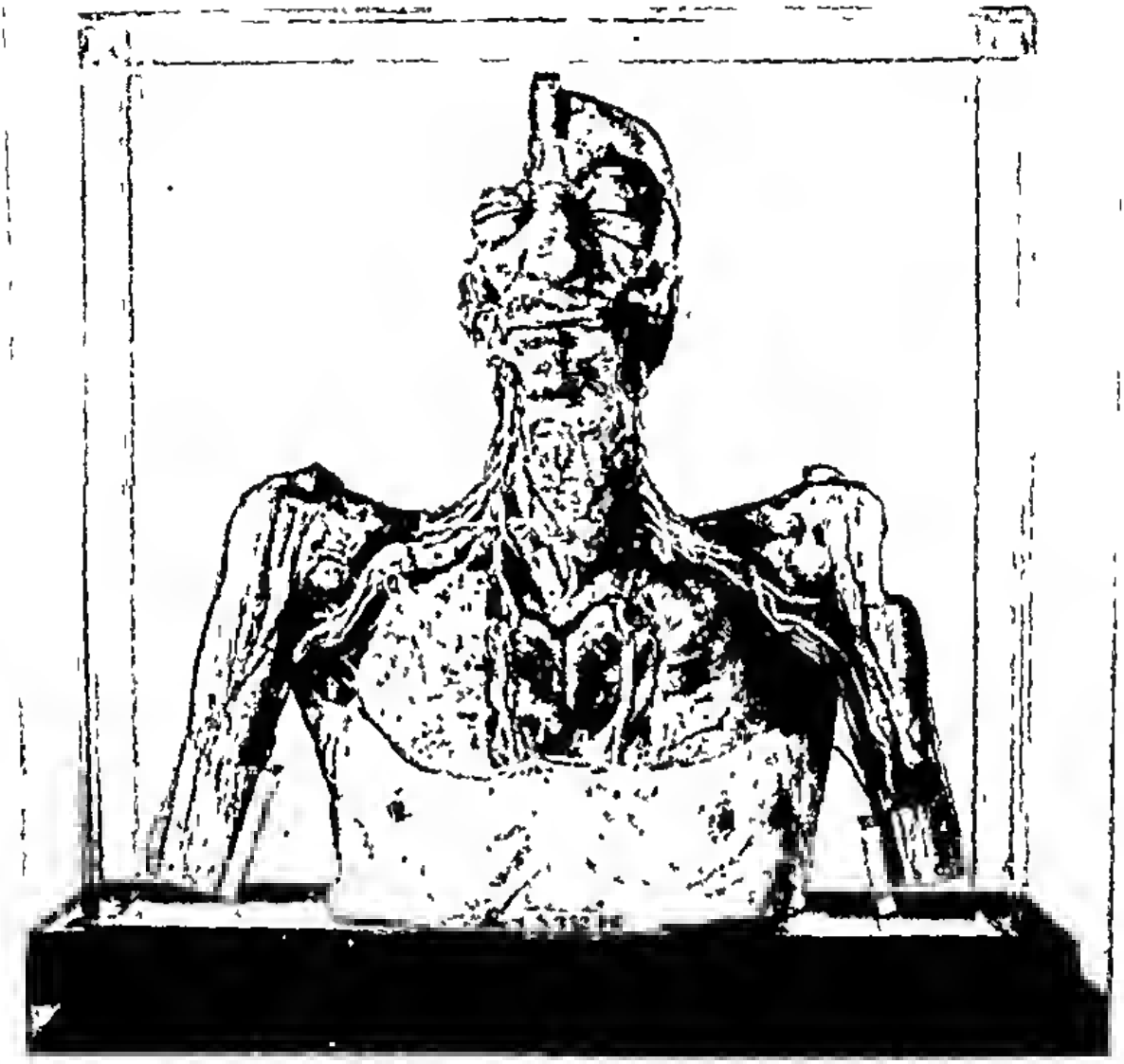

FIG 1

Photograph of a dissection of the head, neck and thorax, to illustrate the very large dissections which it is possible to display in the museum since the advent of Perspex containers The total weight of this exhibit is 196 lb.

increased by the fact that, except in the case of some amputated limbs, the prosector nearly always has to work on very aged cadavers. Delay in obtaining the body after death, and severe arteriosclerosis, frequently increase the difficulties of fixing the tissues, while fatty degeneration of the muscles in old people makes it impossible to produce as clean and neat a dissection as could be made, if cadavers of young people were available.

The advent of Perspex (polymethyl methacrylate) has made it possible to construct in the laboratory rectangular containers large enough and strong enough to contain very large dissections, such as head, neck and thorax, so that now the junctional areas can all be adequately displayed in the museum.

Figure 1 shows a photograph of such a dissection. The whole exhibit weighs 196 lbs.

It is not essential to have an extensive knowledge of anatomy in order to make first class museum dissections, although such knowledge is desirable. The general problems which arise in planning and making a dissection will bear a fairly close comparison with those which arise when a journey is made across a great city like London. For anyone with an intimate knowledge of the great metropolis the journey is usually simple enough, though under difficult conditions it is possible to lose one's way. But those with little or no knowledge of the city can find their way with the aid of a map, though sometimes they may have to ask for directions.

Similarly the planning and making of a dissection is relatively easy for the expert anatomist. Those without this special knowledge must be dependant on an atlas of anatomy, and the guidance of an expert anatomist. And just as it is always slower to find one's way across a city relying on a map, as compared with knowledge of the locality, so the production of a dissection is much slower, if the prosector does not possess a detailed knowledge of anatomy. But it does not follow that the finished dissection will be any less perfect.

It is not enough for those who aspire to become first class prosectors to acquire skill in unravelling the structures of the body. They must also develop the quality of patience which is essential when the dissected structures are being cleaned. The quality of museum preparations depends largely on the skill with which the dissection is cleaned; and this work requires not only much greater skill than the original dissection, but great patience also.

Chapter 2

FIXATION OF MATERIAL FOR DISSECTION

THE fixation of anatomical material involves two processes : it must make the tissues sterile, and it must harden them by coagulating the proteins, so that the various structures are held firmly in place during the subsequent dissection. Excessive hardening of the tissues must be avoided, as this makes the dissection exceedingly difficult.

When only an amputated limb or other part, as opposed to a whole body, is being fixed, quite satisfactory results are obtained simply by injecting the arteries with 5 per cent formalin until the part is moderately turgid. The greatest care must always be taken to avoid introducing air into vessels, as this forms air locks within them and obstructs the flow of fixative. If coloured injection masses are to be injected later, air in vessels invariably spoils the result.

An enema syringe provides by far the most convenient means for injecting all extremities and viscera, both with fixative and with certain coloured masses such as gelatine. The particular advantages of an enema syringe as compared with a piston and barrel syringe are : (1) ease of operation, enabling an almost continuous flow of fluid, since the syringe refills itself automatically (2) the ease with which the injection pressure can be judged, as increased injection pressure is instantly felt as greatly increased pressure required to compress the bulb of the syringe (3) the rapid rate at which, when desired, it is possible to inject (4) the very considerable injection pressure which it is possible to exert, when required, with comparatively little effort.

The artery is connected to the syringe in the following way. A short length of Portex (see Appendix) or similar polythene tubing of suitable diameter is tied into the vessel with linen carpet thread. Anything finer tends to cut into the walls of the vessel. Portex polythene tubing is particularly suitable for tying into all vessels, because, although thin-walled, and therefore possessing a comparatively large lumen, it is very strong, and sufficiently rigid not to collapse when a vessel is tied tightly to it. Its flexibility gives it a further advantage over a glass cannula and, in addition, there is no tendency for a polythene tube to slip out of a vessel, once it has been securely tied in position.

The polythene cannula is connected to the enema syringe by a short length of rubber tubing, of such calibre that the nozzle of the syringe fits firmly into it. If the cannula is too slender to fit the rubber tube, the external diameter of the former is increased by wrapping a suitable length of one inch wide zinc oxide sticking plaster round its end. The roll of sticking plaster is bound with button thread before it is inserted into the rubber tube. The joint is made secure by tying linen carpet thread round the rubber tube. It is not necessary to tie the nozzle of the enema syringe into the rubber tube, provided that the size of the tube is such that the nozzle fits firmly into it. The details of this method of connecting an enema syringe to a vessel are shown in Figure 2.

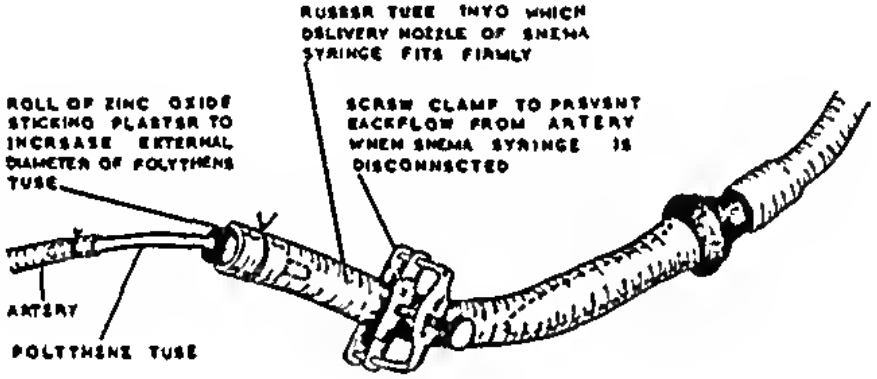

FIG. 2

Method of connecting an enema syringe to a vessel, when injecting with fixative. If the part is to be later injected with a coloured mass, the polythene and rubber tubes are left attached to the vessel after fixation, as they facilitate the attachment of any injection apparatus

The injected material is stored in a tank of 5 per cent formalin until it is required for dissection. Dissection should not however be commenced until at least forty-eight hours have elapsed after the fixing fluid was injected.

When a whole body is being fixed, a rather more elaborate procedure is necessary. The ingredients of the fixing fluid are industrial spirit (95 per cent ethyl alcohol), formalin (a saturated aqueous solution of formaldehyde), liquid phenol and glycerine.

In most departments of anatomy, there is a tendency for a very conservative policy to be followed concerning the exact composition of the fluid used. The quality of the fixed bodies varies considerably. The

properties of each of the substances normally included in the fixing fluid are given below, to help those who wish to modify the formula used.

However, before proceeding to this, it should be emphasised that all serious problems concerning fixation of bodies arise from working in unfavourable climatic conditions. No real problems exist in temperate climates. But if the same formula of fixative, which has proved completely satisfactory in a cool temperate climate, is used in the tropics, fixation may be unsatisfactory. The best remedy is to install air conditioning. It is to be hoped that soon it will be considered as important to have air-conditioned dissecting rooms and mounting rooms in departments of anatomy in all countries which have a hot season, as it is now considered important to provide these conditions in the hotels of air ports where passengers usually spend only a single night.

Spirit is unique among fixing agents for its capacity to diffuse directly through tissues. Consequently if a clot of blood prevents spirit injected into the arteries from reaching a certain area directly, it may reach this place subsequently by diffusion. Spirit has the additional advantages that it does not produce excessive hardening of the tissues, and does not damage the hands or give off an unpleasant vapour from the body during dissection. The objections to spirit are that it tends to bleach the material fixed in it and, during dissection, material fixed only in spirit tends to dry up very rapidly, and so has to be moistened at frequent intervals to avoid damage.

Formalin is an excellent fixing agent, provided that the arterial injection reaches all areas, but its powers of diffusing through the tissues are extremely poor. If injected in too great concentration it produces excessive hardening. It has an unpleasant effect on the hands, though this can be almost completely eliminated by application of suitable barrier creams such as Innoxa B.W.2. (see Appendix). When material fixed only in formalin begins to get a little dry, formaldehyde vapour, equally unpleasant to the lungs and eyes, is given off. In the case of a relatively small part such as a limb, this can be avoided by immersing the material in cold water for a few minutes whenever sufficient formaldehyde is given off to cause discomfort, but in the case of a whole body remedial action is not so easy. Consequently the amount of formalin included in the fixing fluid should be relatively small.

The inclusion of phenol serves three useful purposes. When the phenol reaches the skin it produces whitish patches, which give a clear indication as to how far the injection has travelled. Phenol stains the muscles brown, thus compensating for the bleaching effect of spirit. Provided that only a

moderate amount of phenol is included, the brown tone of the muscles is very pleasing, but excess of phenol stains the whole of the material dark brown. Phenol also helps to sterilize the tissues.

Glycerine is added to reduce the tendency of the material to become dry during dissection. For a rather fat body less glycerine is required than for a thin one.

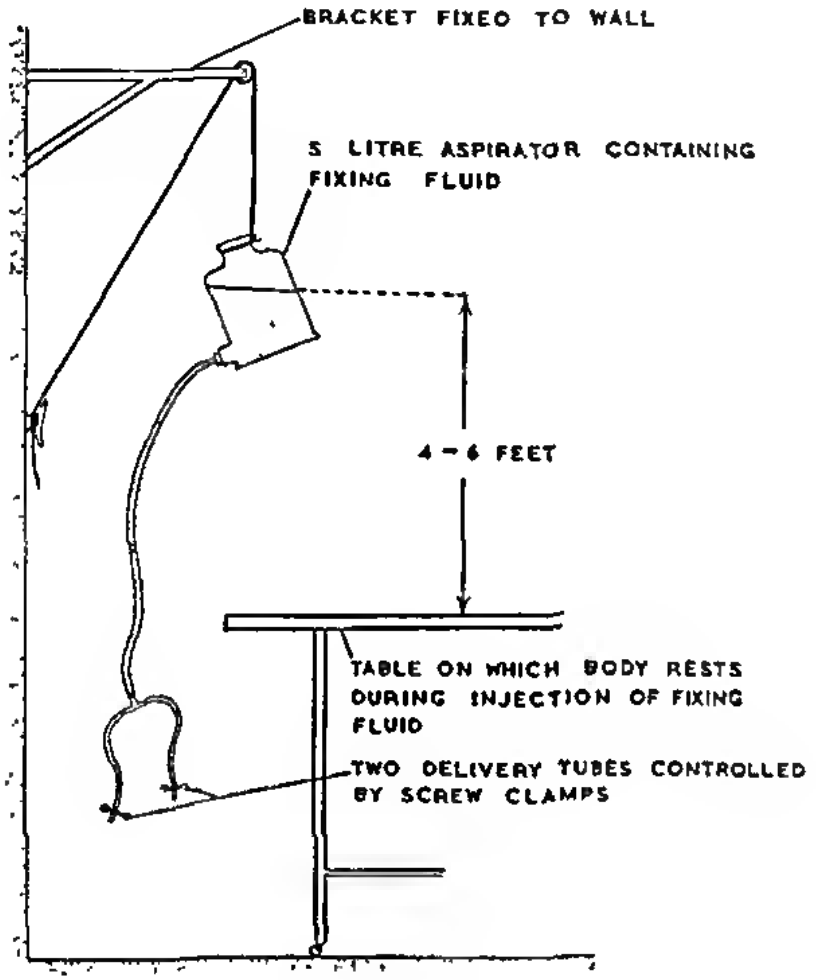

FIG 3

Diagram of the apparatus used for injecting the arteries of the body with fixing fluid

During the injection of the fixing fluid the body is normally laid on its back on a post mortem table, but if it is intended later to make a dissection of some special area such as the perineum, the body must be arranged in the desired position before fixation is commenced. If it is attempted to alter the

and shoulders, on which the body rested during the injection. Whenever practicable dissection should not be commenced for at least a month. The material will remain in excellent condition for dissection (in temperate climates) for several years.

(Note : Material intended for museum dissections should not be stored in phenol, because it tends to stain all the tissues a uniform brown colour. But 2 per cent phenol is preferable to formalin for storing bodies intended for medical students' dissections, because of the high concentration of formaldehyde vapour given off when a large number of parts stored in formalin begin simultaneously to become a little dry during dissection).

position after the injection has been completed, and the tissues consequently hardened, the muscles may be badly torn.

The injection is made in the following way. The femoral artery is exposed in the femoral triangle of one leg. A slit is made in the artery and two cannulae, consisting of short lengths of a suitable size of Portex polythene tubing are tied in the artery, one directed towards the trunk, the other towards the foot. The injection is made by means of the apparatus shown in Figure 3, the two delivery tubes being attached to the cannulae. *Great care* must be taken to prevent any air from entering the femoral artery, as this is probably the commonest cause of the injection failing to flow into one of the upper extremities, owing to the production of an air lock in the axillary artery.

For normal subjects in a temperate climate the following formula is recommended for the injection fluid. Equal volumes of spirit, phenol, glycerine and water are mixed together. (Although phenol does not readily dissolve in water, it dissolves instantly when the spirit is added).

Between eight and twelve pints of this fluid are run in, according to the size of the body, from a height between four and six feet. The lowest height is used from which the flow is reasonably rapid. If during the injection a considerable volume of fixing fluid escapes from the mouth, this leakage is stopped by plugging the mouth and nostrils with cotton wool. Whitish patches on the skin indicate the extent to which the injection has travelled. Flow into the extremities can sometimes be initiated by massage.

After twenty-four hours a further six pints of fluid, similar to that first used, but to which 5 per cent formalin has been added, are injected. It is frequently found that when this second injection is made, the fluid reaches parts to which it did not extend at the first injection. If the injection still fails to reach some areas, these must be fixed by injecting fixative directly into them by means of a hypodermic syringe.

Next a small hole is drilled into the top of the skull in the mid line, and about 100 ml. 10 per cent formalin are slowly injected into the subarachnoid space by means of a hypodermic syringe, to harden the brain. If this fluid is forced in too rapidly, the brain may be damaged. About 100 ml. of 10 per cent formalin are also injected into the pleural cavities on each side.

After the injection of fixative has been completed, the body is placed totally immersed in a tank of 5 per cent formalin. This not only prevents the body from becoming dry, but also, by supporting most its weight, allows the fixative injected into the arteries to reach those parts, usually the buttocks

and shoulders, on which the body rested during the injection. Whenever practicable dissection should not be commenced for at least a month. The material will remain in excellent condition for dissection (in temperate climates) for several years.

(Note : Material intended for museum dissections should not be stored in phenol, because it tends to stain all the tissues a uniform brown colour. But 2 per cent phenol is preferable to formalin for storing bodies intended for medical students' dissections, because of the high concentration of formaldehyde vapour given off when a large number of parts stored in formalin begin simultaneously to become a little dry during dissection).

Chapter 3

COLOURED INJECTION MASSES

1. INTRODUCTION

ALTHOUGH there are a few references to anatomical injections as early as the sixteenth century, it was not until Harvey had established the doctrine of the circulation of the blood in 1628 that this technique was widely used in physiological and anatomical investigations.

The student who seeks a detailed account of the development of this method is referred to F. J. Cole's scholarly account of *The History of Anatomical Injections:* but as the skill and ingenuity of these early workers during the period between 1650 and 1800 has certainly never since been surpassed, and seldom equalled during the subsequent 150 years, their work deserves some mention here, even in a practical text book which deals with current methods. However, the only injection masses with which the present writer is concerned are those suitable for filling vessels sufficiently large to be easily visible to the naked eye, which can therefore be displayed in museum specimens. Injection masses suitable for physiological investigations and histological work are outside the scope of this book.

J. Swammerdam (1637-1680) was the first anatomist regularly to use injection masses which solidified, so that permanent preparations of injected and dissected animals could be produced. Owing to the great cost of spirit and the glass jars needed for wet preparations, many injected specimens were dried and varnished, instead of being mounted in fluid. Consequently injection masses, made up of wax, resin, tallow and turpentine, which are particularly suitable for the preparation of dry specimens, were those chiefly used in the early days. Later, when the cost of preserving wet specimens decreased, injection masses of starch, plaster of Paris, and gelatine came into general use.

The principal drawback of wax injection masses is the difficulty of warming the body or part to be injected sufficiently to prevent premature setting of the wax before the vessels are completely filled, owing to the relatively high melting point and low specific heat of these wax masses. To overcome this difficulty, not only was the body immersed in hot water, but

hot fluids were frequently run into the vessels to warm them up, before the wax injection was thrown in. Care was needed to avoid overheating of the vessels, as this was found to make their walls so brittle that they ruptured during the injection of the wax. From earliest times the desirability of using the bodies of young people for arterial injections, because of the greater elasticity of the walls of their arteries, was widely known.

Starch and plaster masses possess the advantage that they are used cold. On the other hand, they form brittle masses which tend to crumble and are liable to clog up the injection apparatus, so that they cannot be recommended. Gelatine is still regarded as one of the most satisfactory substances for general injections.

Although the methods of some early workers were practised with great secrecy, those of others were described in detail. The earliest general treatise on this subject in English is that of Pole (1753-1829), but no clearer and more concise instructions could be desired concerning the anatomical injection techniques generally followed by the pioneers of this art, than those given by that great anatomist, artist and surgeon, Sir Charles Bell (1774-1842) in the introduction to a *System of Dissections, explaining the Anatomy of the Human body.*

For filling the larger vessels Bell recommends among others the following recipe : Tallow 1 lb., Resin 1 lb., Wax[1] 3 oz., Venice turpentine[2] 2 oz., Spirit of turpentine 1 oz. Concerning the injection of the veins he says : " The success of the injection . . . . depends entirely upon their being well washed with warm water, and repeatedly dilated, as they are for the most part foul with coagulated blood, especially in old people."

The vessels of the lymphatic system are exceedingly difficult to fill by direct injection of a coloured mass, because of the minute size of most of the vessels and the fact that the smaller ones cannot be filled via the larger, owing to the presence of numerous valves. Mercury was found to be by far the most suitable substance for this work, on account of its extreme mobility and the ease with which tiny threads of it can be seen with the naked eye. But its weight causes even a comparatively short column of it to rupture the vessels, and it escapes freely from the smallest hole. Any movement of the injected specimen may result in the escape of a considerable proportion of the mercury. Therefore the great labour involved in the preparation of mercury injections of the lymphatics as museum specimens

[1] Presumably bees-wax
[2] Resinous juice of the larch

cannot be recommended, although this method played an important role in the original study of the lymphatic system.

A very clear picture of the technical difficulties involved in the injection of the lymphatics with mercury is given by Sir Charles Bell, who says :

"*OF THE LYMPHATICS.* — The injection of the lymphatic vessels is the most difficult part of practical anatomy. The subject taken for lymphatics, should be under twenty-five years of age and dropsical. — The apparatus in the shops is fit for every purpose; generally however, the tube of glass is too thick, which makes it heavy and unwieldy when filled with mercury. A provision of very fine forceps, scissars, lancets, needles, and thread, should be at hand, and the assistant must be equally adroit with the anatomist. — The mercury must be pure, and the globules leave no tract behind them.

Supposing that an extremity is to be injected : the veins and arteries should be previously injected. It is placed with the upper part of the limb a little inclined downward. The integuments are to be dissected off; the common cellular membrane left; lines will be perceived small as the most delicate nerves, but without their white opacity, and taking a course somewhat obliquely, crossing the cutaneous veins, below the ancle and on the wrist. It will be difficult to introduce the point of the smallest tube unless we proceed in this way. Having discovered a lymphatic, a delicate needle and thread is put round under it; then, with very fine scissars cut the filament half through. The scissars I use in preference to the lancet, as by snipping the lymphatic a little obliquely, an opening is made which is more easily found than a puncture. We may now inflate the vessels by a small blowpipe, making the stream of air play on the punctured part of the vessel; but I never do this. I introduce into the vessel, the delicate steel poker, if it enters smoothly and without resistance, I know that it has found the vessel; if it is pushed on with difficulty, that it is making its way amongst the common cellular substance. When the poker is introduced, I then take the pipe with a high column of mercury and make the stream play along the poker, when the mercury never fails to enter the lymphatic; and now the point of the tube easily enters the distended vessel, when the poker is to be withdrawn.

The mercury should be allowed to flow freely : from one small vessel, on the wrist or foot, six or ten lymphatics may be filled on the thigh or arm. With similar precautions other vessels are to be sought and filled.

If we have to inject lymphatics betwixt the glands and trunk of the

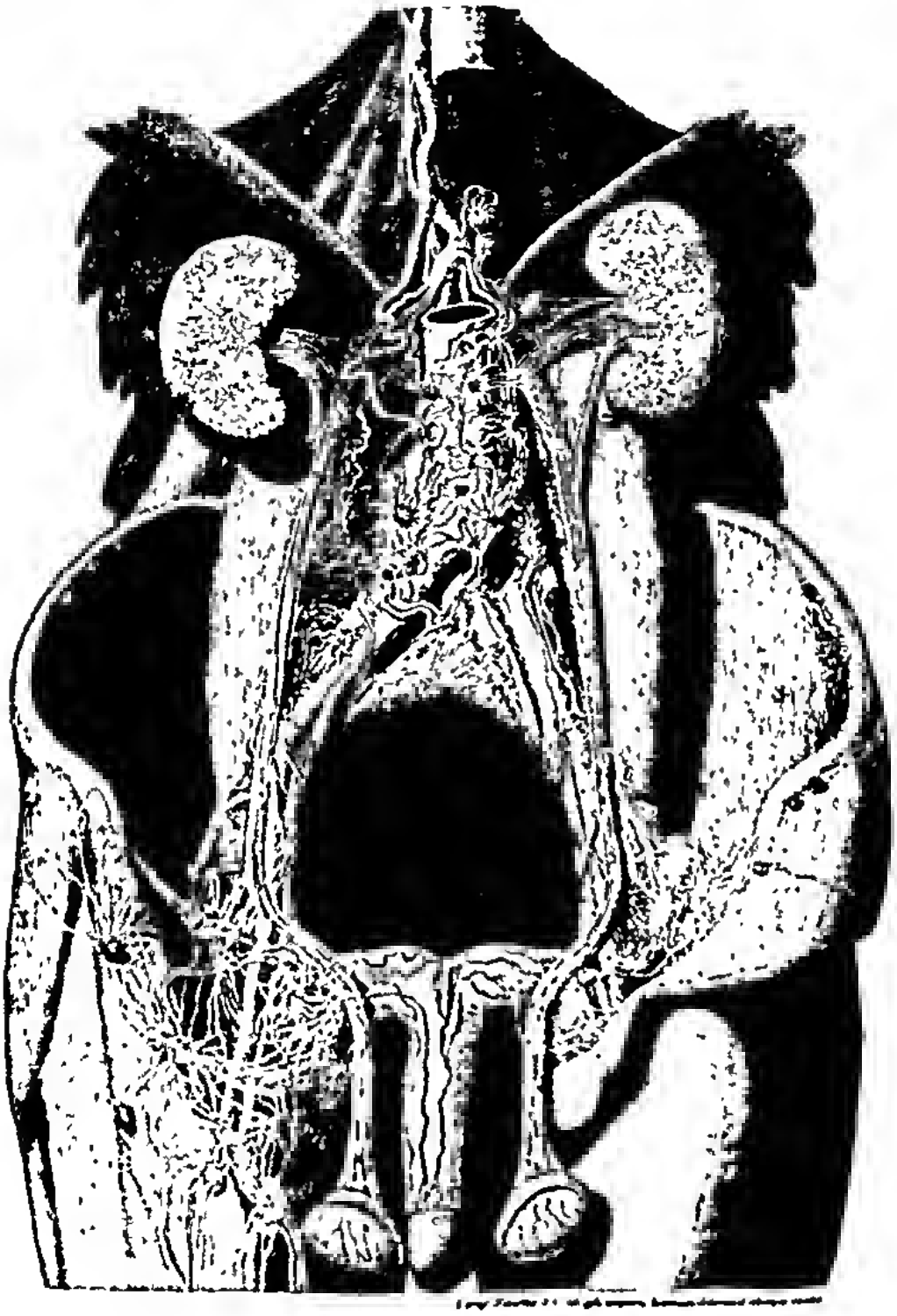

FIG. 4

Mercury injection of lymphatics by P. Mascagni (reproduced from *Vasorum Lymphaticorum Corporis Humani Historia* (1787). Tab. XIII (Semi-)

stem, the pipe may be plunged into the glands so as to fill its cells, from which it will pass or may be pressed into the second set of vessels.

The lymphatics of any subject may be injected. We have them here injected and dissected for the lectures on that subject, in whatever body may offer in the rooms at that time, but when there is much fat the dissection of them is difficult, and to preserve them it is absolutely necessary that the subject shall be thin, and anasarcous.

When the limb is injected, it should be laid horizontally. We begin the dissection on the lower part; when we have dissected the vessel to some extent, a very fine thread is tied round it, and this ligature is repeated as we proceed up the limb, at the distance of five or six inches. When a vessel of, perhaps, three feet in length, is left without this interruption to the mercury, it cannot be expected that when the vessels dry, and the valves consequently shrink, the coats of the vessels will bear so high a column. It will always be remembered that it is the height of the column, not the quantity of mercury in the tube, or in the vessels, which governs the force with which it presses at the lower part."

The greatest exponent of all time of the art of injecting lymphatics with mercury was P. Mascagni (1752-1815). Figure 4 shows an illustration of one of Mascagni's preparations.

By the end of the eighteenth century a fairly accurate and complete picture had been gained of the principal lymphatics by means of the method of direct injection of mercury. But by this method it was not possible to fill the very fine vessels, owing to the physical impossibility of inserting cannulae into them, and to the presence of valves which prevent them being filled from larger vessels.

A new technique was devised by V. Fohmann (1794-1833) by which lymphatics, too small for the insertion of a cannula, could be filled with mercury by an indirect method.

Fohmann made a horizontal incision just beneath the surface of the part to be injected, which was usually either skin, mucous membrane or serous membrane, (he had little success with muscles) at such a depth that the ends of the network of lymphatics were severed. He placed a cannula in this incision, and while holding the flap of tissue in its original position with his fingers, ran in some mercury. Then he pressed the surface over the pool of mercury with the handle of a scalpel. If the incision had been made at exactly the right depth, the mercury flowed into the cut ends of the lymphatics until it reached the glands. It was easy to observe whether the

mercury had by accident entered blood vessels, as it flowed so readily in the latter that it invariably reached vessels large enough to be recognised as such. Fohmann found that the best results were obtained when the mercury was introduced immediately after the termination of rigor mortis, or alternatively into material preserved for some time in spirit. Figure 5 shows an illustration of one of Fohmann's injections.

Fohmann's work clearly pointed the way to the random puncture technique in which the mercury was introduced by piercing the tissues with a hypodermic needle.

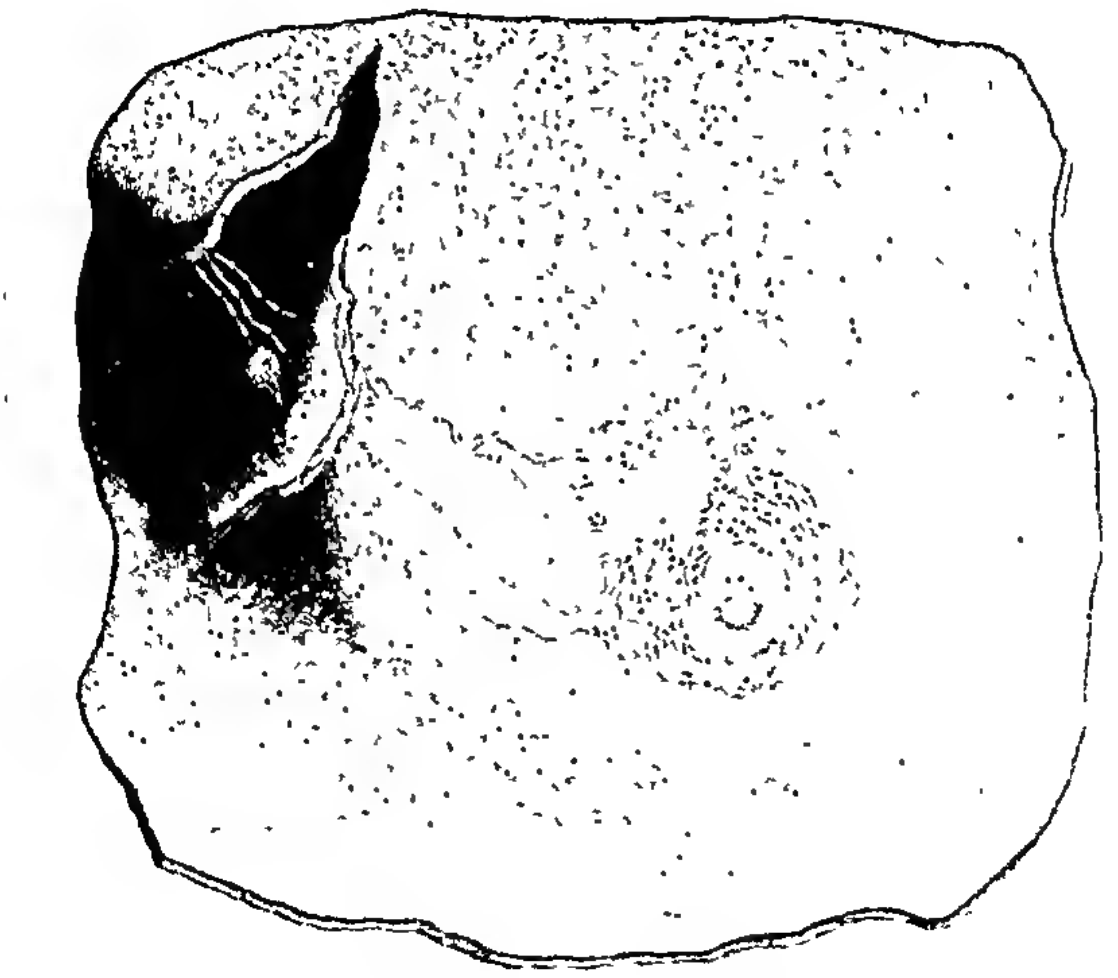

FIG. 5

Mercury injection of superficial lymphatics in the region of the breast, by V. Fohmann (reproduced from *Memoire sur les vaisseaux lymphatiques de la peau* (1833) Plate 1 (Large))

Gerota in 1896 showed that the random puncture method could also be used with a fluid consisting of turpentine and ether coloured with Prussian blue and other pigments, and that by this method both small and large lymphatics could be coloured, without staining the other tissues. Preparations made by Gerota's technique have the added advantage that they can be dissected without the injection mass running out of the cut ends of vessels, as happens with mercury injections, and they can be preserved in formalin.

Since the publication of Gerota's work, modifications of his technique have been used almost exclusively to demonstrate the lymphatics; but the value of this technique is greatly reduced by the fact that the material must be injected as soon as possible after death and at the very latest before rigor mortis sets in.

The details of methods used by earlier workers to demonstrate the lymphatics have been described in some detail, as the present writer has had no experience of this highly specialised and exceptionally difficult work.

2. MATERIALS AND METHOD

There are three requirements for a satisfactory injection mass. It must be sufficiently fluid for easy injection; it must set fairly soon after injection; and after setting it must either be flexible or, if rigid, sufficiently strong to resist fracture while the dissection is being made.

The properties, method of injection and relative merits of three masses: gelatine, latex and synthetic resin will be considered.

General Procedure. In the case of an amputated limb the coloured injection of arteries or veins can be made immediately after the injection of the fixative, provided that the limb is squeezed with the fingers to press out enough of the fixative to make room for the coloured mass. The filling of the vessels of a whole body should be undertaken about forty-eight hours after the second injection of fixative (see Chap. 2). During this interval the fixative has had time to permeate to all areas, and sufficient fluid has escaped, partly via the apertures of the body, and partly by evaporation from its surface, to make room for the coloured mass. But if the coloured injection is made much more than forty-eight hours after fixation, its flow may be obstructed by the coagulated blood which is beginning to form hard clots. However, successful arterial injections can sometimes be made at least five days after the completion of fixation.

The injection of the arteries is a simple operation. The fixative has

already washed them free of blood, and their capacity is so small that when the coloured injection is thrown in, the fixative displaced readily diffuses into the surrounding tissues. Unless the walls of the arteries have become brittle as a result of severe arteriosclerosis, an injection pressure considerably higher than the blood pressure during life can be safely applied without fear of rupturing a major vessel, though care must be taken to avoid extravasation from small vessels, which would stain the surrounding tissues and thus spoil the appearance of the subsequent dissection.

The injection of the veins is a much more difficult operation. First they must be washed out to remove as much as possible of the blood with which they are invariably filled after death. In the case of an amputated limb much of the blood can be washed out during fixation by injecting 5 per cent formalin made up in normal saline, instead of water, into the arteries. If the formalin is made up in water, the part soon becomes waterlogged; but if it is made up in saline, before this happens a considerable amount of fixative escapes via the cut ends of the veins, washing out much of the blood. Washing out the blood by injecting normal saline alone is not recommended, as the part is liable to become so waterlogged, that it is difficult subsequently to inject enough formalin to ensure satisfactory fixation. The washing out of the veins is completed by injecting fixative into small veins at the end of the extremity, and allowing it to escape from the openings at the point of the amputation.

If it is intended to fill the veins of the trunk with a coloured mass, the femoral and internal jugular veins are opened before the arteries of the body are injected with fixative. A very much larger quantity of fixative is run in than for normal fixation, so that the surplus which escapes from the openings of the great veins removes as much as possible of the blood.

Owing to the thin walls of the veins, a lower injection pressure must be used when the veins are being filled, than in the case of arteries, to avoid the risk of rupturing the walls of the former. Also a much larger volume of injection mass is required to fill the veins than the arteries. To facilitate the flow of the coloured mass into the veins, the fixative displaced is allowed to escape through openings in the great veins until the coloured mass also begins to escape. Then the openings are closed by means of artery forceps.

In the case of extremities, the filling of the veins with a coloured mass is complicated by the presence of valves. Consequently a satisfactory venous injection of the arm or leg can only be made by injection from a vein at the distal end. so that the flow of the mass is not impeded by the valves.

hardens gelatine considerably, rendering it at the same time quite insoluble, and it no longer melts when heated.

The injection is carried out in the following way. The material to be injected is warmed up to about 30°C by immersion in a water bath. The gelatine mass is maintained at a temperature of approximately 40°C, and is injected with an enema syringe (see text p. 6, and Fig. 2, p. 7 for method of attaching enema syringe to the vessel to be injected), except in the case of large veins, when the large volume of gelatine to be injected, and the low injection pressure required, make it simpler to run the gelatine in by gravity flow.

In the case of arteries the best indication as to when the correct amount of gelatine has been injected is given by the considerable increase of pressure required to compress the bulb of the enema syringe. A sudden reduction in the pressure required indicates that a major vessel has ruptured, and further injection will result in extravasation into the tissues. A further indication of the extent of the injection is usually given by a flush which appears on the skin when the gelatine reaches the fine vessels just beneath the surface.

When veins are being filled, relatively gentle injection pressure must be used. The degree of distention and turgidity of the injected veins gives the best indication as to how much of the mass should be thrown in.

Gelatine-injected material is first placed in cold water to set the gelatine, and is then kept in a tank of 5 per cent formalin. It must not be kept in spirit, as this partially dehydrates the gelatine, causing it to shrink and crack.

If the primary injection is incomplete, it may be completed during dissection with the aid of a hypodermic syringe. Before a local injection is made into an incompletely filled vessel, all the surrounding tissues which have already been dissected are painted with uncoloured gelatine solution, which is allowed to set, and is hardened by immersion in formalin overnight. This treatment prevents the coloured gelatine used to top up a vessel from soaking into the surrounding tissues and staining them, if some escapes from the cut ends of the vessel being injected. To reduce to a minimum leakage of gelatine used for topping up vessels, it is injected when it is on the point of setting. If it sets in the cannula of the syringe, it is remelted by immersing the cannula in hot water. Gelatine which escapes on to the dissection is allowed to set. Then most of it can be easily dissected away in lumps, any remaining traces being washed away with hot water applied with a soft brush, before it has had time to be rendered insoluble by the formalin in the dissection.

The most suitable cannulae for fixing into both arteries and veins for introducing injection masses consist of short lengths of Portex or similar polythene tubing. (See text p. 6, and Fig. 2, p. 7 for details of the method of connecting the cannula to the injection apparatus).

Gelatine injection mass. This is prepared by mixing 25 g. powdered gelatine with 100 ml. cold water, and heating over a water bath until the gelatine is completely dissolved. While the gelatine mass is being heated, and while it is waiting to be used, the receptacle containing it must be covered to prevent the formation of a skin on the surface of the gelatine solution.

For colouring the mass, insoluble pigments, in the form of fine powders, rather than soluble dyes, must be used, as the latter will in time diffuse completely out of the gelatine. Chemically stable pigments giving an intense colour, and composed of comparatively fine particles which separate readily when added to the gelatine solution, should be selected. Sufficient pigment is added to ensure that even a slender filament of gelatine is intensely coloured so that the gelatine injection will show up clearly in quite small vessels.

From the earliest times Vermilion (mercuric sulphide) has been recognised as an ideal red pigment for injection masses. Prussian blue has been widely used for colouring blue masses owing to the intensity of the colour and the minute size of the particles, but it is not suitable for anatomical preparations, as it is chemically unstable, and fades in time when exposed to light. Boston blue colour dispersion (see Appendix), supplied by the manufacturers for colouring latex, is also suitable for colouring gelatine. This dispersion is supplied as a concentrated aqueous suspension and a comparatively small quantity of it, when added to the gelatine mass, gives the latter an intense blue colour. This pigment appears to be chemically stable, but has not yet been tested for this work over a long period of years. Other Boston colour suspensions can also be used for colouring gelatine injection masses.

If the gelatine is not required for immediate use, 0.1 per cent of thymol crystals are dissolved in it while it is hot, to prevent the growth of moulds. The gelatine can then be kept indefinitely in an air-tight preserving jar, and melted down when required.

A 25 per cent solution of gelatine melts at approximately 28°C, but does not become really fluid until its temperature reaches about 35°C. It should not normally be used in greater concentrations than that recommended above, as a stronger solution absorbs so much water after setting, swelling in the process, that there is the risk that if a very concentrated gelatine solution is injected into large thin-walled vessels, their walls may be ruptured. Formalin

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There are several advantages attached to the use of gelatine as an injection mass. It sets rapidly and the setting time can be controlled in two ways, either by altering the temperature at which the mass is injected, or the temperature of the material being injected. It is extremely simple to use, no elaborate apparatus being required for making the injection. It is also clean and pleasant to handle, and the mass can be prepared and stored, so that it only needs warming up before use. It is particularly suitable for filling large veins and large arteries, but the greatest advantage of a gelatine injection mass is the facility with which incomplete injections can be topped up during the course of the dissection. Once the gelatine has been hardened in formalin, it is quite durable, showing no tendency to crumble and fall out at the ends of the vessels filled with it.

The principal drawbacks to its use are the time required to heat up sufficiently the material to be injected to ensure that the gelatine will not set in the vessels before the injection is complete, and the comparative fragility of slender filaments of the mass, which consequently provide little support for slender vessels filled with it.

Latex Injection mass. This consists of a very fluid aqueous solution which can be coloured as desired. The solution is kept alkaline by the addition of ammonia. When it becomes even slightly acid it sets into a highly elastic rubbery mass.

A number of types of latex are available. One particularly suitable for arterial and venous injections is Boston Neoprene latex (see Appendix). This can be obtained in various colours, and aqueous colour suspensions can be added if necessary, to intensify the colours.

When latex is injected, it must contain sufficient ammonia to prevent premature coagulation within the vessels before the injection has been completed. Even when material fixed only with formalin is injected with latex, the carbon dioxide present in the preserving fluid will in time cause the latex to coagulate; but when the fixing fluid also contains phenol, coagulation takes place sooner.

The general practice is to add sufficient ammonia to the latex so that it smells quite unmistakably (but not strongly) of ammonia, and to inject it as swiftly as possible, by means of pressure exerted by air or any neutral gas. The swift injection reduces to a minimum the risk of the latex setting before the injection has been completed.

Figure 6 shows the apparatus used for latex injection. It is necessary to have a completely reliable means of holding down the cork of the bottle

or jar containing the latex, as the delay caused if the cork comes out during the injection may result in some of the latex first run in coagulating before the rest can be injected. A cylinder of oxygen is suitable for providing the injection pressure. The regulator valve is set at the desired pressure, with the delivery tube filled with latex, but with its end clamped. For arterial

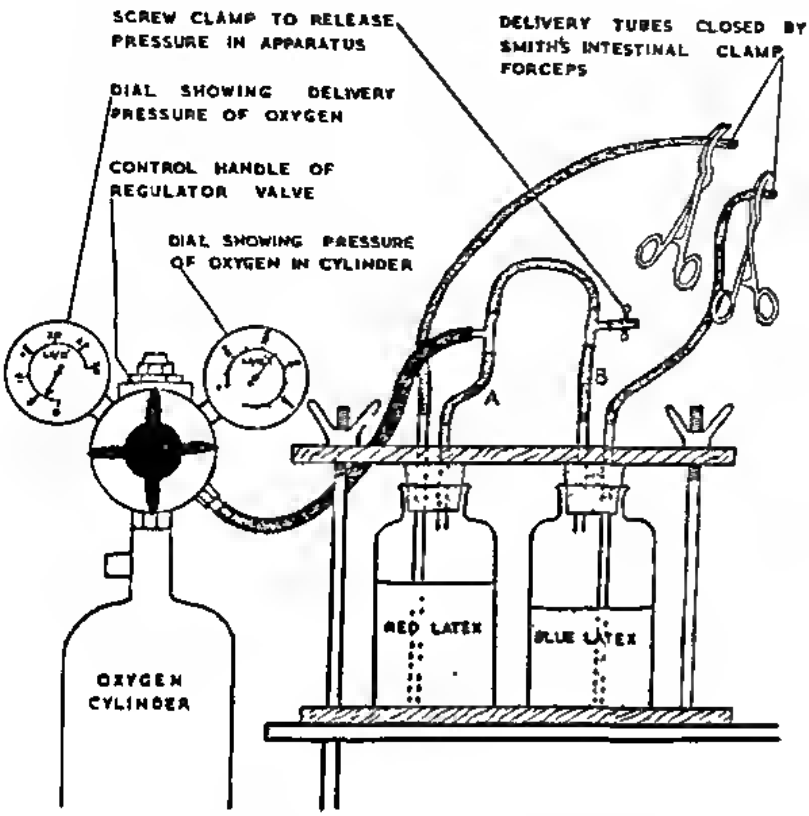

FIG. 6

Diagram of the apparatus used for the injection of latex Note the simple but secure method of holding down the rubber bungs of the jars containing the latex Smith's intestine clamp forceps are used for closing the delivery tubes, as they are less liable to cut into the rubber than artery forceps If only one colour is being used, the rubber tubing is also clamped at either point A or B

injections a pressure of not more than 7 lb. per square inch for a whole body, and not more than 4 lb. per square inch for a limb, should be used. For venous injections the minimum pressure which will cause the latex to flow is used.

The clamp at the end of the delivery tube is only removed for the minimum time necessary to allow the vessels to be filled. If excessive pressure is used, or if the pressure is allowed to act on the latex in the vessels after the latter are filled, extravasation of latex into the tissues is likely to occur, owing

into the tissues, if the comparatively fragile walls of the veins are broken before the latex has set. No information is yet available concerning the long term durability of latex injections.

Synthetic Resin Injection Mass. Synthetic resin produces a rigid cast of the blood vessels, but except in special cases it is not suitable for filling vessels of an internal calibre of less than one mm. diameter, as the setting of the resin is somewhat inhibited by contact with water. The special advantage of using synthetic resin is that the walls of the vessels can be stripped completely away during the subsequent dissection, leaving a brilliantly coloured cast of the cavities of the vessels, which is strong enough to be self-supporting. The practical information concerning this use of synthetic resin will be found in Chapters 16 and 17.

In conclusion it may be said that, speaking generally, latex is the most suitable mass for filling arteries, but gelatine is more satisfactory for the larger veins. Synthetic resin is particularly suitable for filling the veins of an extremity.

REFERENCES

Bell, C. (1810) *System of Dissections, explaining the Anatomy of the Human Body*, 2nd Ed., London.

Gerota, D. (1896) Zur Technik der Lymphgefassinjection; *Anat Anz.*, 12, 216-224

Pole, T. (1790) *The Anatomical Instructor;* London.

Cole, F. J. (1921) The History of Anatomical Injections In *Studies in the History and Method of Science*, ed Singer C., 2, 285-343.

to the fluidity of the latex, and the comparatively long time it takes to solidify.

Before the arteries of a limb are injected with latex, a few small cuts are made at the end of the extremity, and the injection is stopped as soon as latex appears in the cuts. The vessel by which the injection is made must be securely tied before the injection tube is removed, to prevent latex escaping. Any other large vessels from which latex escapes must also be ligatured, but small leaks can be checked by applying 10 per cent acetic acid with a wad of cotton wool. The acid coagulates the latex and seals the leak.

If the arteries of a whole body are to be filled, a pint of a solution made by adding one part of strong ammonia to ten parts of cold water is injected immediately before the latex, as a final precaution to prevent premature setting of the latex in some of the vessels. Between 1½ and 2 pints of coloured latex are run in, according to the size of the body. If an injection pressure of 6 lb. per square inch is used, the injection of the latex usually takes between ten and fifteen minutes.

No exact data can be given concerning the time the latex takes to coagulate, as this depends on several factors, the chief of which are:—the amount of ammonia in the latex, the amount of acid in the tissues of the material injected, and the calibre of the vessels injected. In the case of very large vessels, although a fairly thick layer of solid latex may form quite soon round the outside, the latex at the centre of the vessel remains liquid for a much longer period. Consequently care must be taken during dissections in which there are very large vessels filled with latex, to avoid rupturing a vessel while there is still liquid latex within it, as this would cause the dissection to be badly stained. In order to determine whether or not the latex is completely coagulated, the vessel is gently squeezed in one place, while it is held at another point about two inches away. If the latex is still partly fluid, pressure exerted at one point is felt at the other.

There are two outstanding advantages of latex as compared with gelatine for vascular injections. The material to be injected with latex does not have to be warmed up. This is an important consideration when a whole body is being injected. Also the toughness and elasticity of latex facilitates the preservation of small vessels as, even if the walls of the latter are torn during the dissection, the latex within holds the vessels together.

Latex cannot easily be used for topping up incomplete injections, owing to its fluidity and the delay in setting. It is not suitable for filling very large veins, because of the delay in setting and the risk of extensive extravasation

into the tissues, if the comparatively fragile walls of the veins are broken before the latex has set. No information is yet available concerning the long term durability of latex injections.

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Pole, T. (1790) *The Anatomical Instructor*; London.

Cole, F. J (1921) The History of Anatomical Injections In *Studies in the History and Method of Science*, ed. Singer C., **2**, 285-343

Chapter 4

DISSECTING INSTRUMENTS

A COMPARATIVELY small number of instruments are required to make first class dissections, but it is essential always to use those suitable for the particular job. Only the very best quality instruments are satisfactory; *e.g.* forceps which grip firmly and are easy to hold, scissors which cut cleanly, and scalpels with really sharp edges.

It is not intended here to describe every instrument which may be needed on special occasions; but it may be helpful to those undertaking this type of work for the first time, if something is said about the instruments with which most of the work is done.

These instruments will be considered under four headings : (1) Scalpels, (2) Scissors, (3) Forceps and (4) Miscellaneous Instruments. With the exception of the Paragon handles and blades, the instruments recommended and illustrated are made by Allen and Hanburys Ltd. (see Appendix), and for the convenience of those who may desire to obtain identical instruments, the current catalogue numbers are included.

Scalpels. The most satisfactory scalpels for general work consist of handles to which expendable blades are fitted. Paragon handles (see Appendix), together with those blades which are most suitable for general dissection, are illustrated in Figure 7. Blade No. 20, which fits handle No. 4, is suitable for all but the finest work. The special advantage of this blade is the sharp curvature of the cutting edge, which enables the latter to be brought to bear very easily, with the handle held in a wide variety of positions. For delicate work blade No. 15, requiring handle No. 3, is recommended, while on those occasions when either a fine sharp point, or a straight cutting edge is required, blade No. 11 is used.

These blades are very soon blunted. They can be resharpened by stropping them on the palm of the hand. This can be repeated two or three times before it is necessary to replace the blade.

Scissors. Figure 8 shows three pairs of scissors, adequate for all general requirements. The straight, blunt, seven-inch scissors are used for cutting anything tough, such as a tendon, which would damage a more delicate pair. The straight, sharp, five-inch scissors are used for trimming and cutting

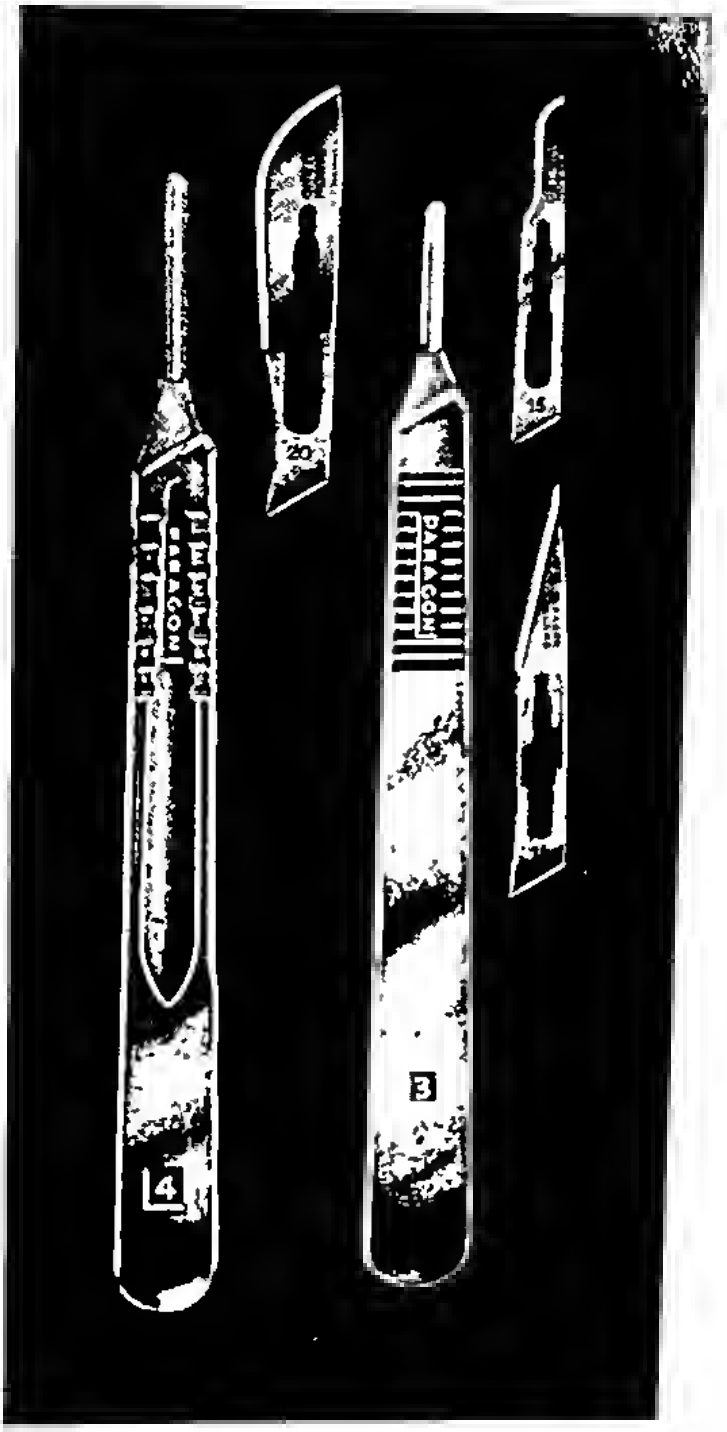
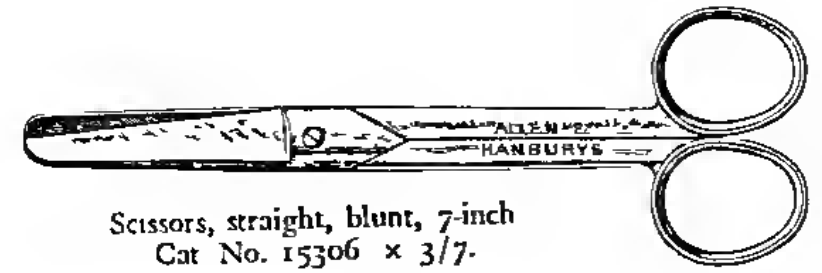
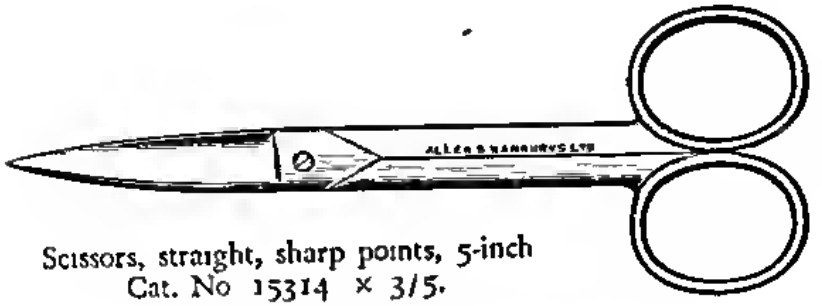


Fig. 7

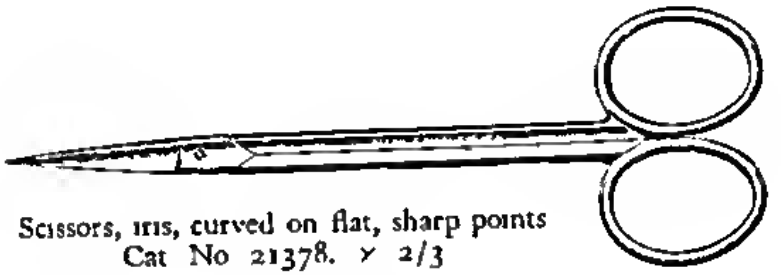
Paragon scalpel handles and blades × 1



Scissors, straight, blunt, 7-inch
Cat No. 15306 × 3/7.



Scissors, straight, sharp points, 5-inch
Cat. No 15314 × 3/5.



Scissors, iris, curved on flat, sharp points
Cat No 21378. × 2/3

FIG. 8

Dissecting Scissors.

generally. For all delicate work the long curved iris scissors are used. The curved blades enable the cutting edges to be brought to bear in places which would be inaccessable to straight scissors.

Forceps. A great deal of dissection is done the blunt way, *i.e.* by pulling instead of cutting, and consequently dissecting forceps are exceptionally important instruments. They must have the following characteristics. The spring which separates the two halves must not be so strong that the fingers become tired by pressing them together. The serrations of the ends must be fairly large, and fit well together, so that they grip securely. The points must be fairly fine, to facilitate picking up small pieces of tissue, and the handles must be of a convenient length. For general dissection two pairs of McIndoe dissecting forceps, illustrated in Figure 9, are strongly recommended, as they embody all the above-mentioned characteristics, and in addition

McIndoe dissecting forceps. Cat. No 15162. × 2/3

Wallis dental dressing forceps Cat. No 27600. × 2/3

Iris forceps, straight, serrated ends Cat No. 21116 × 1.

FIG. 9

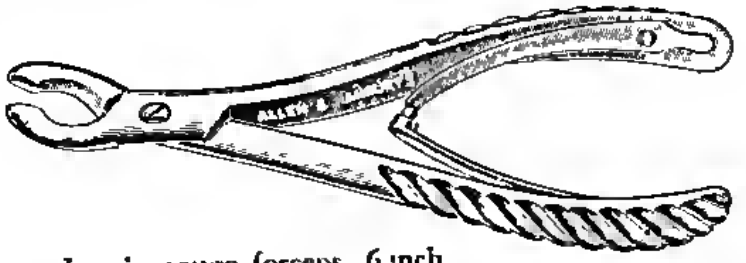
Dissecting Forceps

possess grooved handles which make them particularly comfortable to hold. Wallis dental dressing forceps, also shown in Figure 9, are useful for getting into places which would be inaccessible to straight forceps, while for very fine blunt dissection, two pairs of the iris forceps shown in Figure 9 possess the characteristics of the McIndoe forceps on a smaller scale. It is important that the comparatively coarse serrations at the ends of the iris forceps interlock very well, so that delicate strands of tissue can be firmly grasped. Before buying iris forceps, it is advisable to examine the serrated ends with a hand lens, to ensure that the particular pair chosen is satisfactory in this respect.

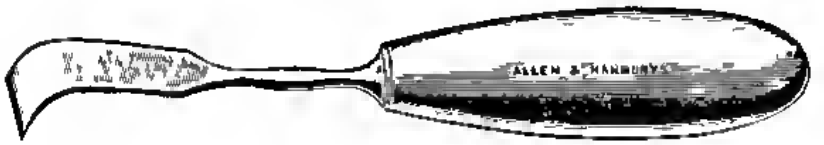
Miscellaneous instruments. Although the safest way of cutting neatly through bone is by means of a saw, or with a dental burr attached to the flexible arm of a dental lathe, there are occasions when pieces of bone can be removed without risk of damage to the dissection by means of bone-cutting forceps. For general work a seven-inch straight pair of these forceps, shown in Figure 10, are suitable, as they can be used for comparatively delicate work as well as for cutting through large pieces of bone.

Luer's gouge forceps, also shown in Figure 10, are ideal for tearing away the remains of muscles and ligaments, and sometimes pieces of periosteum, from bone. This is the best instrument to use whenever an exceptionally firm grip on some tissue is desired, as they grip soft tissues without cutting through them, and are far more effective than ordinary forceps.

Bone cutting forceps, straight, 7-inch Cat. No 40280 × 2/5



Luer's gouge forceps, 6 inch
Cat. No 25006 × ½.



Tudor Edward's rib raspatory Cat No 29158 × ½

Straight, sharp pointed bistoury Cat No 14554 × 1.

FIG 10

Miscellaneous Instruments

The Tudor Edward's rib raspatory shown in Figure 10 is a convenient instrument for scraping away the periosteum from any bone. Its shape enables a firm grip to be maintained without too much effort. For general work the width of the scraping edge may be somewhat narrowed by grinding.

For scraping small recesses in bone into which the raspatory cannot be introduced, an ordinary No. 4 steel scalpel is suitable. As it is mostly the point of the scalpel which is used, it soon becomes blunt, so that it is necessary to have at hand an oil stone on which it can be resharpened. Paragon scalpels are quite unsuitable for this work, as the blades easily break and are almost instantly blunted when used in this way.

For such work as trimming a large cut surface of muscle or fat, a long and very sharp knife is needed. Figure 10 shows a straight, sharp-pointed bistoury suitable for this work. The cutting edge should be carefully honed and stropped before use.

Chapter 5

PLANNING A DISSECTION

THE greatest obstacle which confronts anyone who desires to make first class museum dissections is the difficulty of obtaining material in really good condition. Most of the cadavers available to the prosector are those of very old people, in which there is either fatty degeneration or wasting of the muscles. The former condition makes the production of a really clean dissection impossible, while dissections made from material in which the muscles have wasted do not provide an adequate representation of normal anatomy. It is therefore important to select the very best of the available material especially for large dissections which may involve several months' work.

The next step is to ensure that the body or part is fixed in the most suitable position. It must be placed in the desired position before the tissues are fixed as, if it is attempted to alter the position afterwards, some muscles may be torn. Sometimes it is only necessary to allow the material to rest in the desired position during fixation; in other cases it must be securely held in place. For example, for dissections of the hand, the fingers must be straight and spread out. To fix the hand in this position the wrist and fingers may be tied with tape to a sheet of Perspex in which slots have been drilled at convenient places, through which to pass the tape. Tape is used in preference to string in order to avoid either cutting into the skin, or impeding the flow of the fixative by excessive local pressure. If a dissection of the perineum is contemplated, the body is fixed in the lithotomy position, and if the axilla is to be dissected, the arm must be suitably placed before fixation.

The next point to consider is whether it is desirable to fill the vessels with a coloured mass. If the veins are to be filled, some modifications of the normal fixing technique are necessary to ensure that as much as possible of the blood is washed out (see p. 19). If it is intended to preserve the veins they should always be injected; otherwise their appearance in the finished dissection is very unsatisfactory, as some parts of them are usually greatly distended with blood clots, while others are empty and contracted. Furthermore their walls are so thin and flexible that they *do not* hold their original

position well, unless supported by an injection mass. On the other hand, injected veins are so large that they tend to obscure other structures such as arteries and nerves, so that, except in special circumstances, it is not satisfactory to display veins in dissections in which it is intended to demonstrate the arteries and nerves. However, in the case of a dissection of a bilateral part, this objection can be overcome by removing the injected veins on one side.

In deciding which injection mass is the most suitable, several points must be considered. Coloured latex, which is ideal for injecting the arteries, is not satisfactory for filling the veins of a whole body, owing to the delay in the coagulation of the large volume of latex required, and the risk of extravasation, which results not only in some of the vessels becoming empty, but also causes the tissues to be stained. But although latex has so much to commend it for arterial injections, it cannot be selected if it is desired to commence the dissection immediately afterwards, owing to the delay in coagulation.

Synthetic resin should not be used to fill the vessels of material intended for making a dissection of the head and thorax, owing to the risk of this comparatively rigid mass being fractured through movement of the head during subsequent handling of the part. If the resin is broken it may split the walls of the vessel concerned.

It is best to restrict the plan of a dissection to very general terms. As the work progresses, it gradually becomes obvious that by leaving certain structures and removing others, or by cutting windows in them, the value of the dissection will be increased. It must be remembered that, unlike the dissected material used for demonstrations in the dissecting room, the museum dissection cannot be handled to improve the view of deep structures partly obscured by superficial ones But if when a deep dissection is made the superficial structures are completely removed, it is impossible for students to appreciate clearly the depth of the region displayed. Consequently superficial structures should be removed only when it becomes obvious that their presence seriously impairs the view of the deeper parts which it is desired to demonstrate. Whenever practicable, a margin of skin should be left around the dissected area, as this not only gives a neater appearance to the finished dissection, but also provides a further indication of the depth of the various structures.

When a very large dissection is being planned, the order in which the various areas are dissected must be arranged in such a way that the risk of a part previously dissected being accidentally damaged during the dissection of another area is reduced to a minimum.

Before a dissection of the head, neck and thorax is commenced, the pleural cavity between the collapsed lungs and the thoracic wall should be filled with gelatine solution. This is made up by soaking leaves of bone gelatine sheet, 120 bloom, in cold water until they are fully saturated, and then melting the surface-dried leaves over a water bath. The part is stood on its head while the gelatine is poured in, and held in this position until the gelatine has set. The pleural cavities should be filled before the dissection is commenced, as the thoracic wall may be pierced during the dissection, causing leaks through which the gelatine would escape. If the cavities are not filled with gelatine, when the specimen is placed in the Perspex container and the container is filled with mounting fluid, the large amount of air trapped within the specimen may make it so buoyant that its stability is affected. In the case of a smaller specimen, air trapped in this way can be released by inverting the container, but a container large enough to hold a head and thorax cannot be inverted when filled with mounting fluid without risk of an accident, owing to its great weight.

Chapter 6

THE TECHNIQUE OF DISSECTION

ASSUMING that parts of the body are available in good condition and properly fixed, the first requirement of the museum prosector is really good light in which to work. A good north light (in the northern hemisphere) is preferable to windows through which the sun sometimes shines, but it is essential to have a suitable source of artificial light as well. This is provided by an adjustable electric lamp stand, of sufficiently heavy design to take a lamp of about 150 watts. The lamp stand must be adjustable, so that the lamp can be brought quite close to the dissection when delicate work is being done, and so that the source of light can be moved about, to prevent the hands and instruments from casting a shadow on the area being dissected.

An exceptionally suitable light is provided by 160 watt Philips blended lamp (see Appendix). This lamp combines a mercury arc with an incandescent element, but, unlike most mercury arc lamps, it is connected direct to A.C. mains. Apart from the restful white light provided by this lamp, it gives out much less heat than an ordinary incandescent lamp of similar power, so that it may be placed quite close to the work without the heat causing rapid drying of the dissection. As these lamps are rather expensive and do not behave quite like ordinary electric lamps, full details concerning them should be obtained from the makers.

The second requirement is a comfortable chair of adjustable height. Museum dissection is an exacting and fatiguing occupation if it is done really well, and to reduce fatigue to a minimum, the height of the chair should always be adjusted according to the height of the area under dissection above the level of the work bench, so that the most comfortable posture is achieved.

The third requirement is a pair of magnifying prismatic spectacles. These not only reduce the risk of eye strain and delay the onset of fatigue, but enable the prosector to recognise small structures more readily than with the unaided eye, and thus give him warning when it may be necessary to dissect with special caution. Only a small amount of magnification is desirable for general work. Speera binocular spectacle magnifiers (see Appendix), giving magnification of 1.75 diameters, are particularly suitable for this type of work. (See Figure 11). They are very light, and so can be worn without discomfort for

FIG 11

Pair of Speera binocular magnifying spectacles. The positions of the lenses can be easily adjusted according to the distance between the eyes

long periods. Their lenses can be adjusted according to the distance between the wearer's eyes, and there is a working distance of about nine inches. The design of these glasses is such that, when searching for instruments, or looking at any object more distant than nine inches, the eyes can look over the top of them, so that they can be worn continuously during dissection. For exceptionally fine work, similar spectacles giving magnification of 2.5 diameters are also useful, though the smaller field of vision and the shorter working distance make the more powerful spectacles unsuitable for general work.

As much dissection is done with each hand holding an instrument, and both hands in action at the same time, it is essential that some means be devised to hold the part under dissection firmly in the required position. A number

of lead weights, made by hammering one foot lengths of heavy grade lead pipe until they are flat, serve this purpose well. The weights can be bent as required.

The importance of using only dissecting instruments which are both suitable for the job and of first class quality has already been stressed. These instruments must be treated in such a way that they remain in first class condition.

The greatest enemies of the museum prosector are impatience and fatigue, the two usually marching hand in hand. In a large and complicated dissection such as that illustrated in Figure 1 (p. 4) which takes up to three months to complete, serious damage may result from half an hour's careless or hurried work. It is therefore advisable to stop dissecting as soon as any symptoms of impatience or fatigue are recognised. All those who specialise in this work know that this advice is exceedingly difficult to put into practice, as a feeling of impatience creates a strong desire, not to stop work, but to press on.

Before describing the actual technique of dissection, it may be helpful to give a general picture of the work which has to be done. The various structures of the body, comprising bones, ligaments, muscles, glands, arteries, veins, lymphatics and nerves, are wrapped up in connective tissue, or fascia, strands of which attach each structure to its neighbours, and to the skin. In certain areas, particularly just beneath the skin, and in certain localities such as the ischio-rectal fossa, there are usually large quantities of fat, lying in loculi, which are incompletely separated from each other by strands of fascia. The firmness with which one structure is bound to another varies greatly in different parts of the body. For example the skin is attached quite loosely to the underlying structures on the back of the hand, so that it can easily be pulled away, but on the palm it is attached very firmly.

The technique of dissection consists first of the removal of the skin and the underlying fat and fascia, so that the deeper structures are uncovered. This operation is complicated by the fact that many nerves and blood vessels, which it may be desired to preserve, lie buried in the superficial fascia.

Next some of the more superficial structures may have to be partially or completely removed, in order to display the deeper ones. All the structures which have been exposed must be carefully cleaned, by removing from their surfaces the fascia in which they are wrapped. This is a difficult and time-consuming task, during which great care must be exercised to avoid damage to the structures themselves.

The importance of cleaning the structures of a museum dissection as perfectly as possible cannot be over-emphasised. Although differences of colour may at first help to differentiate the various structures, this cannot be relied upon, as structures are frequently stained by blood pigment in fixed material, and in any case, after a number of years the tissues usually become more or less bleached. The only reliable way, therefore, by which the different types of structure can be clearly recognised is by their textures. These different textures are fully revealed only when the fascial sheaths which covered the structures have been completely removed.

Before the dissection is commenced, the part is washed over-night in running cold water to remove from its surface the formalin in which it has been stored. During the dissection it is sprayed with water whenever it shows any signs of becoming dry, as once any area has become really dry, the brownish appearance thus produced cannot be completely removed. All areas not being dissected at the moment are covered with cheese-cloth soaked in water, and by waterproof sheeting over the cheese-cloth. Sufficient formalin diffuses from the part into the cheese-cloth to prevent moulds growing on the latter, at any rate in temperate climates. Partly dissected material need be returned to the formalin tank only at the week-end.

The dissection is commenced by first locating as accurately as possible the places where the superficial nerves emerge from among the deeper structures. Grant's atlas is particularly helpful for this, because each figure was drawn from an actual dissection, and the outline of each drawing was traced from an enlarged photograph. Exceptional topographical accuracy has consequently been achieved.

A shallow incision about two inches long is made through the skin, as nearly as can be judged over the place where a cutaneous nerve emerges into the superficial fascia, and in the same direction as that in which the nerve runs. The skin on either side of this incision is then removed for a distance of about an inch, exposing an area of about four square inches of the fascia, which is then dissected away with two pairs of McIndoe's dissecting forceps (see Figure 9), one pair being held in each hand. The fascia is pulled away in small pieces until the nerve is found. If the main nerve is not immediately found, a fine branch may be encountered. This can be distinguished from a lymphatic or fine uninjected artery by the fact that, even when pulled quite firmly, it does not break. If a fine nerve is encountered, its course is followed until it leads to the larger nerve from which it arises. The courses of arteries or veins encountered during this work are also followed.

To avoid any risk of damage to the nerves or vessels which are being traced the following method of dissection is employed. The fascia is gripped simultaneously by two pairs of forceps, as close as possible to each side of the vessel or nerve, at the place where it disappears into the surrounding connective tissue. The two pairs of forceps are then pulled away from each other, so that the strands of fascia are torn. The adjacent fat is picked away, and then the process is repeated. For this work it is necessary to develop equal dexterity with the left and right hands, as both are used simultaneously, and equally. A scalpel is used only when fascia too tough to tear away with dissecting forceps is encountered.

The area from which the skin has been removed is extended, when it becomes necessary, in order to follow the course of nerves or vessels already located. By proceeding in this way, eventually all the skin and superficial fascia are removed, except from those areas where, when the dissection was planned, it was decided to leave a margin of skin in the completed specimen.

Before extending the dissection to a deeper level, the structures previously uncovered are carefully cleaned. This greatly simplifies the next stage of the work. The prosector who does not clean up his dissection as he proceeds, imposes on himself a handicap similar to that produced by wearing spectacles with dirty lenses. His view of the structures is impaired, and this results in inferior work.

The next step consists of freeing the superficial structures which are to be removed, from neighbouring structures to which they are bound by connective tissue. As much as possible of this work is done with the fingers, but care must be taken not to tear the deeper structures. Those most likely to be torn in this operation are muscles. When separating two muscles the risk of tearing the fibres of the deeper one can be greatly reduced by placing a finger, or the flat part of the handle of a scalpel, on the surface of the deeper muscle at the place where cleavage is taking place. This counteracts the tendency for some of the fibres of the deeper muscle to be pulled away.

Whenever a scalpel has to be used to separate two structures, these should be stretched firmly apart, and divided by making a shallow incision, which is then gradually deepened by repeated light strokes with the curved part of a really sharp blade. By proceeding in this way, arteries and nerves come into view before they are cut, so that they may be preserved intact.

Although it is desirable to leave in place any superficial structure which does not obscure the view of the deeper parts which it is intended to display, the prosector must resist the temptation to burrow deeply into dark holes.

When the course of a nerve is being followed, he will often be tempted to pursue it to a depth where it is no longer possible to see clearly what he is doing. This may result in damage to deeper structures which is only revealed later when the more superficial ones are removed.

As far as possible the prosector should always have at hand reliable illustrations of the area on which he is working. Such pictures not only show him what structures he may expect to find, and give him warning about those areas where it is necessary to proceed with extra caution, but they also give a clearer idea than any amount of printed description of the parts being unravelled.

Although accurate illustrations are very helpful, the greatest safeguard against serious accidents is the ability to proceed in such a way that no important structure is severed before it has been uncovered, and to recognise structures the moment they come into view. The ability to recognise instantly small uninjected arteries, small nerves and certain glands, while they are still almost completely buried in fat and fascia, comes only after considerable experience. When an experienced prosector supervises the work of a novice, one quick glance at the dissection in progress is often sufficient for the expert to see the cut ends of several nerves and arteries which the beginner has dissected away through inability to recognise them among the connective tissue and fat in which they were buried. In this respect it should be mentioned that the appearance of the various structures in fixed material is very different from that in unfixed parts.

The dissection of ligaments presents special difficulties because, especially in old people, the actual ligaments are often covered by a thick coat of very tough, whitish, fibrous tissue. The fibres of the ligament itself are distinguished from this fibrous connective tissue by their shiny surface, their much greater tensile strength, and the fact that they cannot be stretched. When searching for ligaments, the connective tissue is picked away in small pieces with dissecting forceps, whenever this method is practicable, but it is sometimes necessary to use a scalpel or scissors as well. When this is done, only very small cuts are made, just sufficient to facilitate the blunt dissection, as unrestricted use of scalpel or scissors can easily result in an important part of the ligament being pruned away. This is particularly likely to happen when the ligaments of the knee and ankle joint are being dissected. This danger is increased by the fact that the figures and descriptions of some of the ligaments of the knee and ankle are inaccurate in many text books.

In order to display clearly the mechanically important ligaments of a

joint, it is necessary to remove the thin and mechanically unimportant capsular ligaments which bridge the gaps between them and enclose the cavity of the joint. These can easily be torn and pulled away with dissecting forceps. By displaying part of the joint itself in this way, a clearer picture is given of the relationships of the special ligaments which it is intended to demonstrate.

When dissections of the head are made, it is usually necessary to remove some of the bone. Although some very beautiful miniature circular saws are available for cutting bone, the prosector usually has to manage with less expensive instruments. The safest method of removing bone is to cut it with dental burrs, attached to the flexible arm of a dental lathe. When drilling an area close to some important structure such as a nerve, which it is intended to preserve, only really sharp burrs should be used, for it is necessary to press a blunt burr very hard against the bone to make it cut, and this may easily lead to an accident. The area being drilled must be frequently washed by pouring water over it, to remove debris which obscures the work.

By far the most tedious part of the production of a museum dissection is the work involved in cleaning the various structures. To clean the whole of a dissection really well, without damage to the structures, requires not only great patience but considerable skill. In unskilled hands the dissection, instead of becoming cleaner, gradually degenerates into a torn and ragged mess. A first-class museum dissection is chiefly distinguished from inferior work by the skill with which it has been cleaned. As the technique by which the different types of structures are cleaned varies considerably, the cleaning of each will be described separately.

Cleaning of muscles. The surface of all muscles is covered by a membrane of connective tissue called the epimysium. Extensions of this membrane, called perimysium, pass into the muscle itself, dividing the contractile fibres into a number of irregularly shaped bundles. The thickness of both the epimysium and the perimysium varies greatly, the thicker parts of the perimysium containing small arteries and nerves. The cleaning of a muscle consists of removing the epimysium and as much of the perimysium as is visible. Great care is required during this process to avoid tearing some of the muscle fibres. The cleaning is commenced by dissecting away a flap of epimysium. This is grasped if possible by the fingers, or by forceps if it is too small or inaccessible to be held by the fingers. Whenever possible fingers are used in preference to forceps, as the former are less liable to tear the epimysium. Next the flap is gently pulled away from the muscle, and in

the same direction as that in which the muscle fibres run. At the same time fingers are placed on that part of the muscle from which the epimysium has just been removed, to prevent fibres from being pulled away from the rest of the muscle. When it is found that the epimysium cannot be pulled away without risk of tearing muscle fibres, owing to the toughness of the perimysium, the latter is cut through with curved iris scissors (see Fig. 8, p. 28). Before cutting through the perimysium it is stretched away from the rest of the muscle somewhat, so that the cut ends spring back out of sight among the muscle fibres. When the epimysium is being removed care must be taken not to damage the arteries and nerves entering the muscle.

Cleaning is completed by picking up with iris forceps (see Fig. 9, p. 29), those ends of perimysium still conspicuous after the epimysium has been removed, and trimming them away with the curved iris scissors. After a muscle has been cleaned, it must be handled very gently, to avoid tearing it or detaching some of the fibres from its surface. Damage may easily result if a jet of water is allowed to play on to a cleaned muscle when the part is being washed after removal from the formalin tank. Similar damage may result if the part rests on a cleaned muscle, while another area is being dissected; but damage from this latter cause may be avoided by resting the area which has been dissected and cleaned on a large pad of wet cotton wool, shaped roughly to fit the dissection. While the other area is being dissected, the part and the pad of cotton wool are moved about as one unit. If an elaborate dissection requiring many weeks' work to complete is being undertaken, the area first dissected should also be impregnated with gelatine, by the method described in Chapter 7, before work is commenced on another area.

Special care is required when dissecting the muscles of the face which are inserted into the skin, and those muscles which arise partly from the deep fascia. The dissection of the muscles of the face is facilitated if the work is done with the part in water, with the water just deep enough to cover the area being dissected. Each bundle of muscle fibres has to be cleaned separately, and great care is needed to avoid tearing these delicate muscles. For this work more powerful Speera magnifying spectacles, giving a magnification of 2.5 diameters, can be used with advantage, as these muscles are so pale that the bundles of fibres are not easily distinguished from the superficial fascia in which they lie. The most important example of a muscle which arises partly from the deep fascia is the upper part of tibialis anterior. When this muscle is being dissected, the deep fascia is either cut away with a very sharp scalpel, leaving a somewhat rough surface composed of the cut ends of the muscle fibres.

or alternatively the fascia is left attached to the muscle. If it is attempted to pull the deep fascia away from the muscle, the latter will be badly torn.

Cleaning of tendons, aponeuroses and ligaments. The ends of many muscles consist of shiny inelastic tendons, while others have some of their surfaces covered by a thin sheet, or aponeurosis, composed of similar shiny white fibres. Although the method by which tendons etc. are cleaned is basically the same as when the epimysium is removed from the contractile part of the muscles, in some places considerable force is required, and cutting may be necessary as well as pulling. When well cleaned, it is found that tendons and ligaments are divided up into bundles of fibres in a similar way to the arrangement of the contractile fibres of the muscles. The visible part of the connective tissue between these bundles should be carefully removed.

Cleaning of bone. In a museum dissection it is difficult to distinguish bone from tendon or ligament, unless the periosteum which covers the bone has been stripped away very thoroughly. In most areas the periosteum can be scraped away fairly easily by means of a raspatory (see Fig. 10, p. 30), but the cleaning usually has to be completed either with the point of a scalpel or a stout needle mounted on a handle. A broach-holder, soldered to a metal handle, is extremely useful for holding all sizes of needles. It is much more difficult to clean those areas of bone to which muscles or ligaments were originally attached. Patient scraping is necessary with the point of a steel scalpel (not a Paragon, as the blades are too flexible for this work and are instantly blunted or broken). The point of the scalpel blade needs frequent sharpening on an oil stone during this work, and care is needed to avoid damage to the bone itself. Sometimes the remains of ligaments can be most easily torn away by gripping them with a pair of gouge forceps (see Fig. 10) which are used here, not for gouging, but simply to grip very firmly.

Cleaning of arteries and veins. The walls of all arteries are covered with a felt-like sheath of connective tissue, called the adventitia. This varies in thickness with the calibre of the vessel. It is only by removing the adventitia that the smooth muscular walls are revealed, by which even uninjected arteries are easily recognised in well-cleaned dissections. In the case of arteries filled with a coloured mass, the complete removal of the adventitia is still desirable, as it makes the colour of the injection mass more conspicuous.

It is easier to clean injected arteries than uninjected ones, as the injection stretches the walls out, and holds them steady during the dissection. In the case of large arteries, a longitudinal incision is made with a scalpel through the

adventitia, which is then peeled off by means of two pairs of dissecting forceps, applied simultaneously to each side of the incision. Remnants of the adventitia are trimmed away by first seizing them with a pair of dissecting forceps and then cutting them away with curved iris scissors. It is sometimes possible to clean small arteries simply by tearing the adventitia away with two pairs of dissecting forceps as, if a longitudinal incision is first made through the adventitia, there is a risk that this may also cut into the muscular part of the artery wall. Particular care is needed where arteries branch, to avoid tearing small branches away from the main artery during the cleaning. This danger is greatest in the case of unfilled arteries, or when the arteries are filled with gelatine, which has hardly any tensile strength and consequently gives little support to the artery. In the case of arteries filled with latex, which has great tensile strength, the risk of damage during cleaning is considerably reduced. If the arteries have been filled with coloured resin, their walls may be with advantage stripped completely away, leaving only a coloured cast of their cavities.

The sheath of adventitia which covers the veins is exceedingly thin, and the muscular wall beneath is also thin and easily torn. Except in the case of the larger veins, very little of the adventitia can be removed without risk of damage to the rest of the vessel wall. When a vein is being cleaned, the adventitia should never be pulled away. The part to be removed is gently lifted away from the wall of the vessel with dissecting forceps, and cut with curved iris scissors.

Cleaning of nerves. Nerves are covered with a tough felt-like sheath called the epineurium. Extensions of this, called the perineurium, enclose the individual bundles of nerve fibres of which the principal nerves are composed. Enough of the epineurium must be removed to reveal the smooth white bundles by which nerves are easily recognised in a well cleaned dissection. The cleaning is best done by tearing away the epineurium with two pairs of dissecting forceps, one held in each hand. The epineurium is grasped simultaneously on either side of the nerve and pulled away. Care must be taken not to separate the individual bundles of which the larger nerves are composed. This is particularly liable to happen, unless special care is taken, in the case of the sciatic and femoral nerves. There is no risk of breaking any but the finest nerves while they are being cleaned, as they have great tensile strength. Care must be taken however to avoid stretching them so much that they appear unnaturally long in the finished dissection and may even have to be cut and ligatured in order to take up the slack.

Cleaning of glands. Glands are enclosed by a sheath of connective tissue

called the fascia propria. Most glands have a typical lobulated structure, and extensions of the fascia propria pass between the lobules. The glands are cleaned by first dissecting away a flap of fascia propria. This is then stripped from the gland by pulling it either with the fingers, or with dissecting forceps. When necessary it is freed from the interlobular fascia by cutting. The cleaning must be sufficiently thorough for the lobulated structure of the gland to be clearly recognisable. Particular care is required when the prostate gland is being cleaned. This gland is enclosed in a very tough capsule, but the gland itself has no clearly recognisable lobular structure, so that there is a danger that, when the capsule is being dissected away, part of the gland may be removed at the same time.

Chapter 7

FINISHING THE DISSECTION

UNDER this heading is included all the work which has to be done to the dissection, after the areas which are to be displayed have been dissected and cleaned, in order to give the specimen as attractive an appearance as possible when it is mounted in fluid in its Perspex container.

If any bones containing marrow have been cut across, the marrow is scraped out, and the cavity originally occupied by the marrow filled with gelatine. This reduces the amount of fat which subsequently contaminates the mounting fluid.

Then all cut surfaces, usually consisting of skin, fat and muscles, are neatly trimmed with a straight sharp-pointed bistoury (see Fig. 10, p. 30). The appearance of a bilateral dissection is improved if the skin incision is trimmed so that it is symmetrical.

Next the dissection is immersed in clean cold water. It is then seen that the surfaces of muscles, tendons, bones, vessels etc., which have been so carefully cleaned, are still festooned with ragged ends of connective tissue. These become conspicuous only when the dissection is placed in fluid, which causes the free ends to float away from the surfaces to which they were adhering. It is impossible to remove all these pieces of connective tissue, but the larger ones must be carefully cut away. This can only be done while the dissection is immersed in fluid. Curved iris scissors (see Fig. 8, p. 28) are most convenient for this work which, although tedious and time-consuming, is worth doing well, as it greatly improves the appearance of the finished specimen. The final cleaning must never be done by pulling the connective tissue away, as this only drags more to the surface and, if persisted in, would eventually displace some of the muscle fibres.

When the final pruning has been completed, the dissection is removed from the water and placed on an enamel meat tray. Any vessels and nerves which have become detached from the structures they supply are sewn securely in position. Chinese twist, consisting of a very strong silk thread, available in various gauges, is suitable for this purpose.

However carefully the final pruning of the dissection has been done, its appearance is still marred by a fluffy covering of connective tissue when the

dissection is immersed in fluid, as even movements in the fluid are sufficient to bring fresh ends of connective tissue into view. In order to preserve the clean appearance which the dissection has while resting on the table, when it is mounted in fluid, its surface is impregnated with gelatine. This cements the tiny ends of connective tissue, so conspicuous while they are floating freely, to the surface of the dissection, where they are almost invisible. This treatment also considerably strengthens those parts of the dissection which might otherwise suffer damage as a result of the frequent eddying of the mounting fluid caused by the specimen being moved.

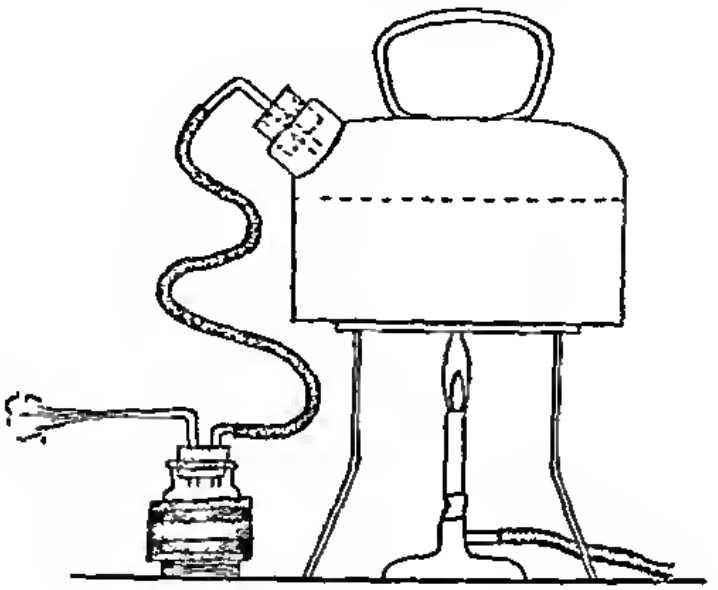


FIG 12

Diagram of a whistling kettle, which has been adapted to give a jet of steam. The whistle has been replaced by a rubber bung, through the centre of which a glass tube passes. A rubber tube leads to a water trap which also serves as a handle when the steam jet is being used. A piece of sponge rubber sheeting is fixed round the water trap, as otherwise it would get too hot to hold

Before the gelatine is applied, the surface of the dissection is mopped with a wad of damp cotton wool. This is used as a sponge, and is pressed firmly against the muscles to absorb surplus water from among their fibres. The cotton wool is frequently wrung out during this work. Water is removed from areas inaccessible to the wad of cotton wool by means of a No. 3 or 4 sable water-colour paint brush. The water absorbed by the brush is removed by squeezing the hairs between the fingers.

Next the whole surface of the dissection is painted liberally with a hot solution of 25 per cent gelatine in water. The gelatine sets almost immediately

FIG 13

Photograph of a finished dissection, from the museum of the Royal College of Surgeons of England, to show the general appearance of a dissection coated with gelatine and mounted in fluid

it comes in contact with the dissection, provided that this work is done at normal room temperature (*i.e.* not more than about 22°C). A jet of steam, which can be conveniently provided by adapting a whistling kettle, as shown in Figure 12, is now played on the dissection. The part on which the steam is directed is held horizontally so that, when the steam melts the gelatine, as much as possible of the latter soaks into the tissues, instead of just running off the surface.

When the whole surface of the dissection has been treated in this way, steam is momentarily directed on to all surfaces, while they are held vertically, to allow surplus gelatine to run off the dissection. But a thin film of gelatine must be left covering the whole surface. Any bundles of muscle fibres which have become partly detached during the course of the dissection are carefully arranged in their original position while the gelatine is still fluid.

As soon as the gelatine has set, the dissection is immersed in cold water and carefully inspected to see whether the whole surface is covered with a film of gelatine. If the steam has removed all the gelatine from any place, ragged ends of connective tissue will be seen, which move when the water in which the dissection lies is agitated. Any such areas are coated again with gelatine.

After this treatment the improvement in the general appearance of the dissection when viewed in fluid is quite spectacular. Although the film of gelatine may be easily seen when the dissection rests on the table, it becomes almost invisible in mounting fluid. Figure 13 shows a dissection treated in the way described above.

After being coated with gelatine, the dissection must be handled with great care, to avoid damage to it. It is stored in 5 per cent formalin until it can be placed in the mounting fluid. The formalin hardens the gelatine somewhat, and alters it so that it is quite insoluble, even when heated.

As mentioned in Chapter 6 (p. 41) when a large dissection is being made, the area first dissected is coated with gelatine before dissection is commenced on the next area, to protect it from damage. But when the dissection has been completed, it is necessary to coat the whole surface with gelatine, including those areas previously treated with it, as the handling which is unavoidable during dissection always causes some of the gelatine applied earlier to come off.

Chapter 8

CONSTRUCTION OF SPECIMEN CONTAINERS AND TURNTABLES

UNTIL fairly recently only glass containers were available for mounting wet specimens. Whenever possible oval or cylindrical jars were used for economy, but these give a grossly distorted view of the dissection. Rectangular glass jars are satisfactory only if the sides are ground flat and polished, and even comparatively small jars of this type are very costly. Consequently containers large enough to hold a head and thorax, or other large specimens were not available.

The advent of Perspex (see Appendix), called Plexiglass in America, consisting of polymethyl methacrylate, has not only revolutionised museum mounting technique, but has freed the prosector from the restriction of keeping the size of his dissections within certain limits.

It is difficult to imagine any material more suitable than Perspex for making museum containers. It can be obtained in large sheets varying in thickness from 1/25 inch to 2 inches. It is easy to cut, machine and join, and its optical properties are excellent. Its toughness and chemical stability make it very durable and, although it is rather easily scratched, it can be repolished without much labour. Spirit, which was used extensively in former times for mounting anatomical specimens, cannot be used in Perspex containers, as it crazes the surface, but formalin and glycerine have no effect on it, and provide satisfactory substitutes.

When Perspex was first used for making museum specimen containers, the joints were made by softening one edge of a sheet of Perspex by soaking it in a solvent such as ethylene dichloride or chloroform, for about a minute, and then bringing the softened edge in contact with another sheet of Perspex. Light pressure was applied to the joint for about an hour, after which time it was strong enough to be handled without risk of damage, though full strength was not developed until after twenty-four hours or longer, according to the thickness of the Perspex used. The solvent was applied by holding the sheet vertically on a horizontal sheet of glass with the edge to be softened kept from actually touching the glass by means of two lengths of fine wire. Then solvent was run from a pipette on to the glass round the Perspex until

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a pool was formed. In order to ensure really good joints free from bubbles, it was necessary to machine fairly accurately the edge to be softened.

After this method had been in general use for some years, it was observed that white patches, of crystalline structure, had developed in many of the joints made in this way. These affect not only the appearance of the containers, but also the strength of the joints, so that in the case of large containers holding a great weight of mounting fluid, there is a risk of them not only leaking, but bursting. It has been established that the most important of the various factors which lead to the development of these defects is lack of precision in machining the edges before the joints are made. Unfortunately really accurate machining is not always possible in the ordinary laboratory, and technicians have found that, as an alternative, it is possible to obtain comparatively good joints by soaking the edge for longer than a minute in the solvent, and then when the joint has been made, applying much greater pressure than recommended by the manufacturers of Perspex. This tends to produce joints in which the subsequent deterioration after a period of about five years may be quite serious. But even with precision work, these defects are still liable to appear sooner or later.

In a museum containing thousands of specimens mounted in Perspex containers, the cost of replacing every container after a number of years would be very great. As there are grounds for believing that the sheets of Perspex will remain serviceable for a great many years, it is worth while to use a method for constructing joints which will also last indefinitely, as then the Perspex containers represent a capital asset instead of a liability: for even if the specimens they contain deteriorate, the jars can be stored for future use.

Fortunately the manufacturers of Perspex have produced a cement, called Tensol No. 3 (see Appendix), which can be used with the ordinary equipment available in the laboratory for making joints which, it is believed, will prove to be as durable as the sheets of Perspex themselves. This cement is supplied in the form of a white powder, consisting of powdered Perspex mixed with a catalyst, and a clear and colourless liquid, composed of Perspex monomer containing a stabilizer. These two ingredients are made up quite easily, by following the manufacturers' instructions, into the actual cement. The viscosity of the cement can be varied by altering the proportion of powder to liquid. For some applications it should be prepared with the consistency of dough, but to prepare cement suitable for making joints by the method described below, thirty parts by weight of the powder are mixed with seven

parts weight of the liquid. This produces a cement just too viscous to flow freely under the action of gravity, but not too viscous to allow a fairly fine worm of it to be extruded from a suitable syringe. If it is attempted to make joints with a more fluid mixture, evaporation of the monomer before the cement sets causes the cement to shrink excessively, producing gaps or bubbles. The special feature of Tensol No. 3 cement, apart from the durability of joints made with it, is its gap-filling property, provided that a sufficiently viscous mixture is used. Consequently, really good joints can be made with less precision work than when they are made by softening the edge of the Perspex with a solvent.

This cement can be conveniently prepared by making about 600 ml. at a time. Immediately after it has been made up, while it is still hot, and consequently sufficiently fluid so that most of it can be poured out of the beaker in which it is prepared, it is transferred to collapsible lead tubes (see Appendix) for storage. Tubes 1½ x 5 inches in size are convenient for this. After filling, the ends of the tubes must be carefully sealed by pressing the metal together, and folding it over twice, to prevent evaporation of the highly volatile monomer. The filled tubes should be stored in a cool place with the delivery nozzle uppermost, so that air trapped in the cement rises to the surface, and can be expelled before the contents of the tube are transferred to the syringe. The cement remains serviceable when stored in these tubes for about a month at a room temperature of 20°C.

When the cement is required, the contents of one of the tubes is squeezed out into a suitable syringe. The syringe used for this work must be comparatively slender, to enable sufficient pressure to be exerted on the viscous cement to extrude it from the relatively small nozzle. The piston must fit well into the barrel, to prevent back flow, and there should be two finger rings at the upper end of the barrel, and another on the handle of the piston, to facilitate the application of the considerable pressure required to force the cement out of the nozzle. The 2 oz. aural syringe (see Appendix) shown in Figure 14 is recommended for this work. It is used in conjunction with a delivery tube made by sawing off the end of an Allen's metal pipe, so that about one inch remains. The diameter of the nozzle can be reduced as required, by tapping the end of the tube all round with a hammer. To reduce the risk of the piston seizing up during use, a little metal is removed from the piston wall, except for about 3 mm. at its lower end. This can be done either on a lathe, or by filing. Sufficient metal should be removed so that the piston slides quite easily throughout the whole length of the barrel.

Also, before the syringe is filled with cement, a thick felt pad is placed inside the hollow metal piston, and saturated with monomer.

After the syringe has been filled with cement, it is allowed to rest with the nozzle uppermost overnight, before use. During this period all air bubbles in the cement rise to the surface. The air is expelled before the cement is used, by pressing the piston in with the nozzle held uppermost. Provided that when not in use the end of the delivery nozzle is covered with a piece of Sellotape, and the syringe is kept in a cool place, the cement remains in

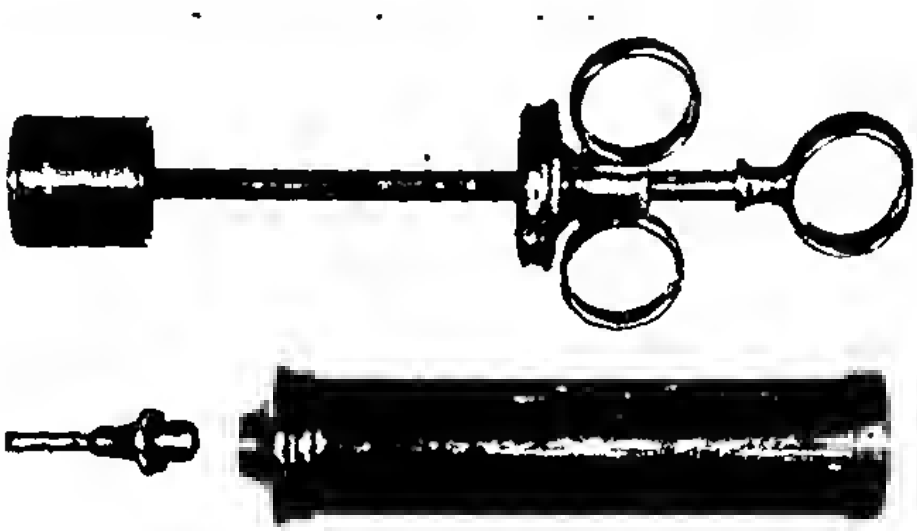

FIG. 14

Syringe suitable for applying Tensol No. 7 cement. It is used in conjunction with an Allen's metal pipe, modified as described in the text. This pipe is one of a number of interchangeable attachments, which can be screwed into the end of the syringe. These articles are obtainable from Down Bros. & Mayer & Phelps (*see* Appendix) 2 oz. Aural syringe, Cat. No. 765 B. Allen's metal pipe, Cat. No. 765 G.

a serviceable condition in the syringe for at least a week. During this period it can be recharged without cleaning. But before the week-end the syringe should be dismantled and left to soak in chloroform, so that it can be cleaned out thoroughly before being used again. If it seizes up when partly filled with cement, it is immersed in chloroform until the piston can be freed; then the syringe is dismantled and cleaned before being used again. Although in skilful hands the syringe should never seize up when in regular use, it is nevertheless advisable to have a spare one in reserve. If it is likely that on some occasions the contents of more than one syringe will be required on the same day, it is advisable to have several syringes already filled, rather than to refill the same syringe and use it again without allowing it to stand overnight. Unless the syringe is allowed to stand for

several hours after being filled, the cement extruded is mixed with air bubbles, which make the application of an even layer of cement impossible.

Immediately before a joint is made, the two surfaces which will come in contact with the cement are painted with monomer, applied with a sable hair water-colour brush. This is most important, as it softens the surface sufficiently to ensure complete bonding of the cement with the Perspex.

In the simplest method of construction, two sides of the container are held vertically in their correct position by means of strips of wood screwed to a wooden base as shown in Figure 15, or by any other means which may be devised. A strip of Sellotape is stuck to the outer surface of the Perspex

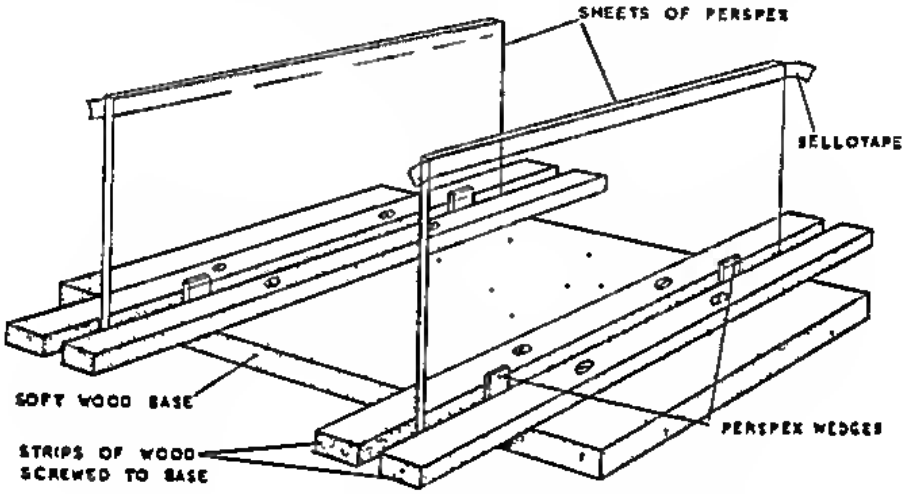

FIG 15

Diagram to show a simple method of holding two sides of a container in position while the third side is being cemented to them.

sheets flush with the upper edges, to prevent cement, extruded when the joints are made, from adhering to the outer surface. The cement is then applied to the two upper edges. During this process, the nozzle of the syringe is held against the edge, and the amount of cement applied is controlled not only by the force exerted on the piston, but also by the speed with which the nozzle is drawn along the edge. Then the sheet of Perspex which will form the third side of the container is placed in position, and light pressure applied by means of weights. In order to obtain neat joints by this method it is essential that a very even layer of cement is applied to the edges; for to ensure a complete joint an excess of cement must be applied, so that some is extruded when the weights are applied. The cement extruded on the outside of the container is removed later, when the construction of the container has been completed. Its removal is facilitated by the presence of the strips of Sellotape, which prevent the extruded cement from sticking to the surface of the Perspex.

They are fixed horizontally, a foot apart, and a reflector, made of chromium-plated metal, is fixed above them (see Fig. 16). A choke is required for each lamp. As the chokes vibrate while operating, they should be mounted on rubber to reduce noise. The joints are placed about eighteen inches below the mercury arcs, and the cement is fully hardened after six hours' exposure. It is advisable to cool the Perspex by means of a fan, or draught of air from an open window, while the cement is being polymerised as, if the cement becomes over-heated by the lamps, bubbles are produced in the joints.

When the first three sides of a container have been cemented together, subsequent work is simplified, as it is no longer necessary to use any special means of holding the Perspex in position while the joints are being made. After the four sides have been stuck together, the top and bottom edges are trued up by taking a thin cut off with a band saw, and then grinding the edges flat on coarse sand paper fixed to a flat surface, such as plate glass, before the bottom is stuck on. While the cement fixing the bottom of the container in place is being polymerised, the open end of the container is stood on two blocks, so that air has free access to its inside. If this precaution is not taken monomer given off from the joint being polymerised may accumulate inside the container in sufficiently high concentration to cause crazing of the surface of the Perspex.

When the container has been completed, its edges are rounded by planing, filing or sandpapering. After this has been done, the cement extruded on the outside of the joints is removed by stripping away the lengths of Sellotape originally placed in position to prevent the cement from adhering to the surface of the Perspex. Finally the edges of the container are polished on a buffing wheel, to which a suitable polish has been applied.

If a vertical milling machine is available, neater joints can be made if a rabbet is cut in one of the two edges to be joined. This reduces the tendency for cement to be extruded on the inside of the joint. In this method the cement is applied to the rabbet (see Fig. 17) instead of to the edge, as in the other method of construction. It is very much easier to apply an even layer of cement to a rabbet than to an edge.

The construction of Perspex containers is facilitated if the sheets of Perspex are stored on horizontal shelves at an even temperature in a fairly dry atmosphere. These conditions ensure that the Perspex sheets are flat when required. If stored by leaning them against a wall, the sheets become warped and this adds to the difficulties of making neat joints. In no circumstances should the piece of Perspex intended to form the lid of a container be used

Cement extruded on the inside of the joints cannot be removed. Provided than an even layer of cement is applied, this extrusion is uniform and hardly noticeable; but otherwise it is uneven, and somewhat spoils the appearance of the completed container.

Next the joint is exposed to light of suitable wavelength and adequate intensity, which stimulates polymerisation of the monomer in the cement, converting it to Perspex. It is important that polymerisation be fairly rapid, to avoid excessive shrinkage of the cement, resulting from evaporation of the

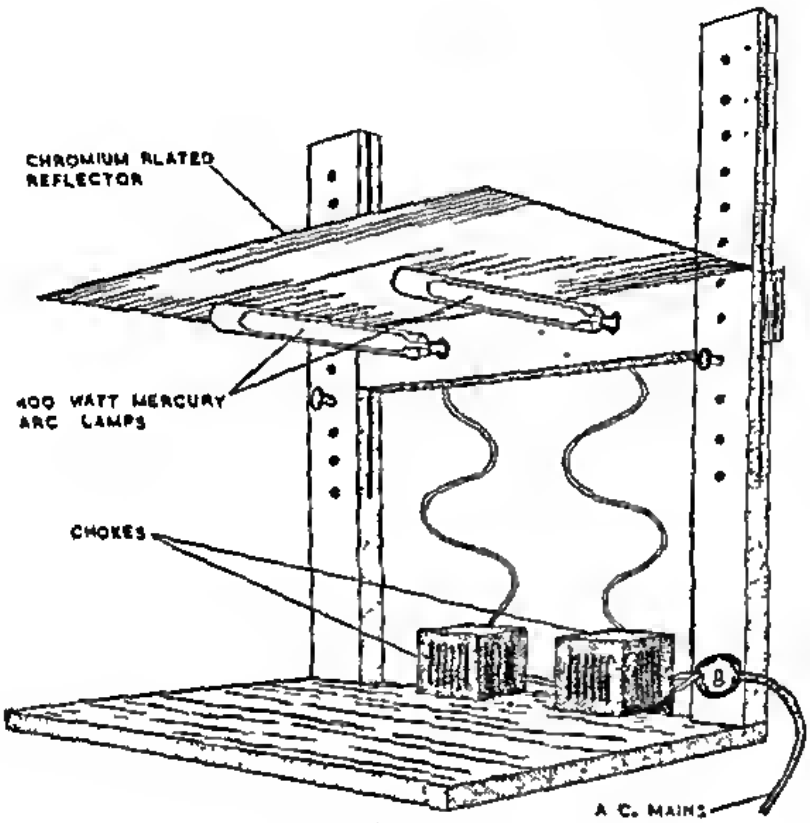

FIG 16

Diagram of the apparatus used for polymerising Tensol No 3 cement in the absence of sunlight.

highly volatile monomer before it is polymerised. The source of light must also be one which does not emit too much heat, as this not only increases the evaporation of the monomer, but may even produce bubbles in the joint.

Bright sunlight is ideal for this work, at any rate in temperate climates. The exposure required varies from two to four hours, according to the prevalent conditions. If it is necessary to use artificial light, an adequate source is provided by two 400 watt mercury arc lamps (see Appendix), of the type used for street lighting. All the ultra-violet waves emitted by these lamps are absorbed by the glass bulbs in which the mercury arcs are enclosed, so they can be operated in the laboratory without elaborate screening.

They are fixed horizontally, a foot apart, and a reflector, made of chromium-plated metal, is fixed above them (see Fig. 16). A choke is required for each lamp. As the chokes vibrate while operating, they should be mounted on rubber to reduce noise. The joints are placed about eighteen inches below the mercury arcs, and the cement is fully hardened after six hours' exposure. It is advisable to cool the Perspex by means of a fan, or draught of air from an open window, while the cement is being polymerised as, if the cement becomes over-heated by the lamps, bubbles are produced in the joints.

When the first three sides of a container have been cemented together, subsequent work is simplified, as it is no longer necessary to use any special means of holding the Perspex in position while the joints are being made. After the four sides have been stuck together, the top and bottom edges are trued up by taking a thin cut off with a band saw, and then grinding the edges flat on coarse sand paper fixed to a flat surface, such as plate glass, before the bottom is stuck on. While the cement fixing the bottom of the container in place is being polymerised, the open end of the container is stood on two blocks, so that air has free access to its inside. If this precaution is not taken monomer given off from the joint being polymerised may accumulate inside the container in sufficiently high concentration to cause crazing of the surface of the Perspex.

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The construction of Perspex containers is facilitated if the sheets of Perspex are stored on horizontal shelves at an even temperature in a fairly dry atmosphere. These conditions ensure that the Perspex sheets are flat when required. If stored by leaning them against a wall, the sheets become warped and this adds to the difficulties of making neat joints. In no circumstances should the piece of Perspex intended to form the lid of a container be used

as a temporary dust cover when the container is filled with liquid, as this soon produces severe warping, caused by the absorption of water on the lower surface of the Perspex sheet. However, warped Perspex can usually be flattened by soaking it in hot water for a period which varies from a few hours to several days, according to the thickness of the sheet, and then allowing it to cool while clamped to a flat surface.

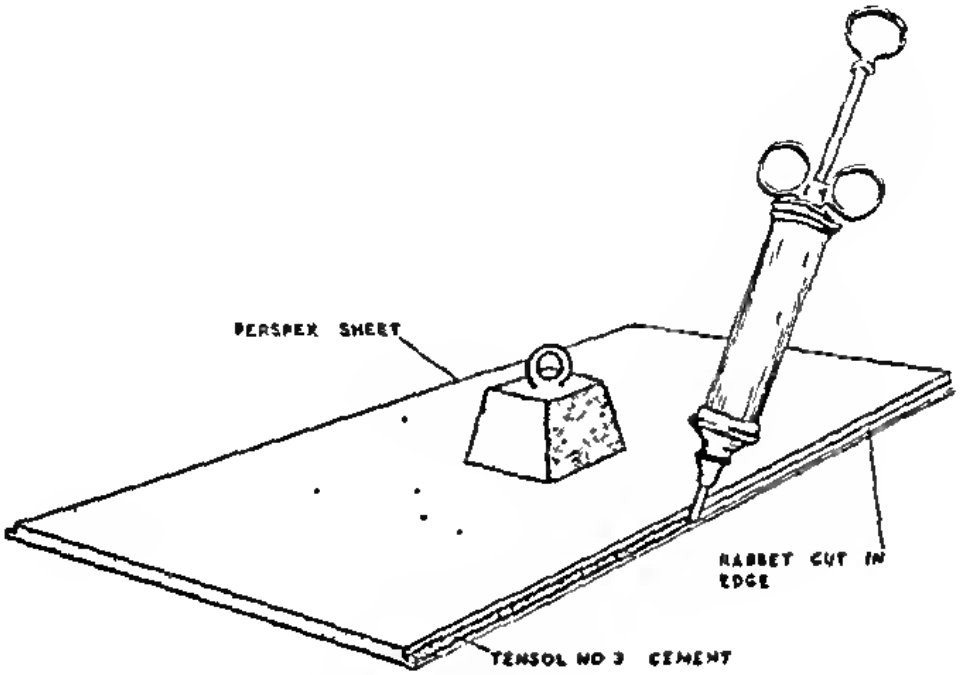

FIG 17

Diagram to show Tensol No. 3 cement being applied to a rabbet cut in the edge of a sheet of Perspex. Note the weight placed on the Perspex to keep it from moving. This frees both hands to manipulate the syringe, but still permits the sheet of Perspex to be quickly moved, if this should be necessary during the application of the cement. The fingers of both hands (not shown in the diagram) may have to be used to press the piston of the syringe down, as considerable force is required to extrude the viscous cement.

Satisfactory joints cannot be easily made with very thin Perspex, even though thin Perspex might be quite strong enough. It is not advisable to use Perspex thinner than ⅛ inch thick. This thickness is adequate for all dust covers except the largest sizes, and for small containers for wet specimens. For medium-sized containers for wet specimens $\frac{3}{16}$ inch sheets are recommended. For really large containers ¼ inch or even ⅜ inch thick sheets should be used, while for the base of an exceptionally large container, such as that used to hold the dissection shown in Figure 1 (p. 4) ½ inch thick Perspex should be used. In each case the thickness of the Perspex selected must be such as will ensure that the container is not only strong enough, but sufficiently rigid to last indefinitely without noticeable distortion.

But unnecessarily thick Perspex should not be used, as this greatly increases the cost.

If more than one side of a part has been dissected, it is desirable to mount the container on a turntable. This not only facilitates the study of the

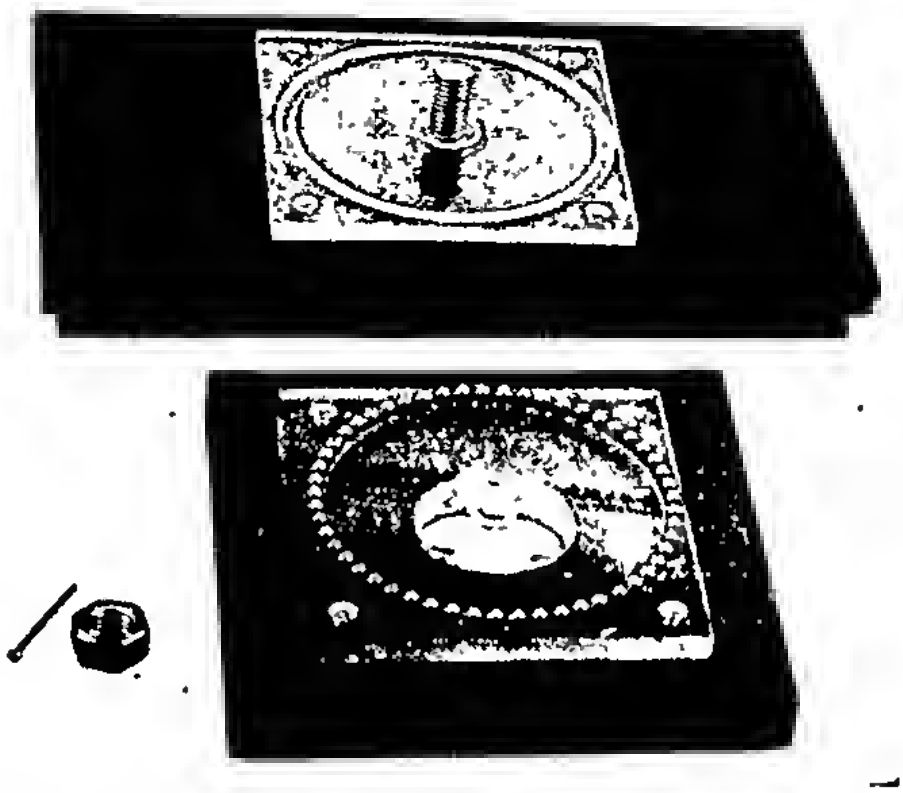

FIG. 18

Photograph of a turntable. The wooden tray on which the specimen stands has been removed and inverted to show the details of construction

dissection, but greatly reduces scratching of the comparatively soft Perspex resulting from frequent handling. Excellent turntables can be made in the following way. Two squares of equal size and suitable thickness are cut from a sheet of Perspex. A half-inch hole is drilled through the centre of each, and a circular groove, of diameter slightly less than the length of the sides of the squares, is cut in each square on a lathe. The grooves are shaped to take nickel balls. One of the squares is screwed to a wooden base, the other to a wooden tray on which the Perspex container will stand. The circular groove on the lower Perspex square is filled with nickel balls of suitable size, and the two squares are held together by means of a half-inch nut and bolt. The nut is held in position by means of a split pin. Figure 18 shows a photograph of one of these turntables with the top part removed and inverted, to show the details of construction.

Chapter 9

MOUNTING OF ANATOMICAL DISSECTIONS

A DISSECTION is mounted in the anatomical position, unless this impairs the view of some of the dissected areas. In the latter case it is mounted so that it presents a view familiar to surgeons.

Dissections mounted in rectangular Perspex containers are held in the desired position by sewing them to Perspex plates. The thickness of the mounting plate depends on the size and weight of the specimen. Sometimes it is necessary to cut a window in the plate through which part of the dissection projects. It may also be necessary to stick one or more projecting rods or strips of Perspex to the plate, either to support the dissection directly, or to provide struts to which parts of the dissection can be tied.

A very heavy dissection, such as the head, neck and thorax illustrated in Figure 1 (p. 4), should be mounted so that it rests on the floor of the container, to reduce the strain on the stitches by which it is tied to the plate.

The dissection is fixed to the plate in the following way. It is laid on the plate and the most suitable points for making stitches are marked on the Perspex with a Chinagraph pencil. The dissection is then removed and pairs of holes to take the stitches are drilled through the plate. Next the marks of the Chinagraph pencil are carefully wiped away, as although they are inconspicuous, and consequently easily overlooked at this stage, when the plate is immersed in mounting fluid, they may become very noticeable.

Bleached linen carpet thread is strongly recommended for sewing all but the smallest dissections to the Perspex plates, as it wears well, does not cut excessively into the tissues, is comparatively inconspicuous, and the knots have no tendency to come undone. Chinese twist, size 0 is suitable for tying small dissections to the plates.

The first knot made to secure each stitch should always be a Granny, as this slips sufficiently when the ends of the thread are pulled, to enable the stitch to be tightened until just the correct amount of tension is produced. Then another knot is tied over the Granny to prevent the stitch becoming loose again.

The plate to which the dissection is sewn is made of such dimensions that it fits easily into the container. The plate is held in position, close to,

but not actually pressing against the posterior wall of the container, by means of pegs, made of Perspex rod, which have previously been cemented to the walls of the container at suitable points by means of Tensol No. 6 cement. There must be a clearance of a least 5 mm. between the top of the mounting plate and the bottom of the lid of the container, and the top edge of the plate must be prevented from actually leaning against the wall of the container by means of a Perspex peg about 3 mm. long. These precautions are necessary to avoid the mounting fluid becoming contaminated by the highly volatile monomer in the cement used to fix the lid of the container in position. Contamination is avoided by very thorough ventilation of the inside of the container while the cement is hardening. Really effective ventilation is impossible if the mounting plate almost touches the lid, and if the upper edge of the plate rests against the wall of the container.

If the mounting fluid becomes contaminated with monomer, the fluid later turns milky after a period which varies, according to the prevailing conditions of light and temperature, from three days to three months. The milky appearance results from monomer, absorbed by the mounting fluid, subsequently polymerising. It necessitates changing the mounting fluid.

In the case of comparatively small exhibits which may be handled and even inverted, a Perspex peg should be cemented into the lid of the container, to prevent excessive movement of the mounting plate in the vertical plane. But before the lid of the container is cemented in position, a careful check must be made to ensure that this peg is not so long that it prevents the lid being placed right down on to the mouth of the container.

The mounting fluid recommended for all dissections mounted in Perspex containers consists of twenty-five parts by volume of pure glycerine, seventy-five parts by volume of distilled water, and five parts of formalin. The glycerine is not essential to preserve the specimen, but improves the optical properties of the fluid. The glycerine and water require very thorough mixing, as the former, being so much heavier than the latter, tends to settle out as a separate layer at the bottom of the mixing receptacle.

The mounting fluid should always be filtered before use. As a large quantity of mounting fluid may sometimes be required if a number of dissections are being mounted, an apparatus is necessary, by means of which the fluid can be rapidly filtered. A simple apparatus, suitable for filtering rapidly a large volume of mounting fluid, is shown on the left hand side of Figure 19. A five-inch sintered glass funnel is used to filter the fluid, and the apparatus is worked by means of a low-power electric vacuum pump, which

can also be used to ventilate the insides of containers while their lids are being stuck on. The sintered glass funnel requires occasional cleaning. The necessity for this is indicated by a noticeable slowing down of the rate at which the fluid passes through it. The funnel is cleaned by immersing it in concentrated sulphuric acid, which is then heated to 80°C. The funnel is then removed and washed. Only those who thoroughly understand the dangerous properties of concentrated sulphuric acid should undertake this work.

Before an exceptionally large container, such as that shown in Figure 1 (p. 4) is filled with mounting fluid, its sides must be supported, to counteract the tendency of the mounting fluid to make the sides bulge (this support is needed only until the lid is stuck on and the container sealed). Adequate support can be provided by tying previously stretched window sash cord as tightly as possible round the upper part of the container, and then inserting a number of wooden blocks between the cord and the Perspex. New sash cord is stretched before use by doubling it round some very firmly fixed object and pulling it hard several times. Some care is needed, as if the cord breaks it could lead to an accident. It is advisable to place any container which weighs more than 100 lb. when full of mounting fluid, on a wooden stand before the fluid is poured in, and not to lift it off the stand while full of fluid. This precaution avoids subjecting the container to severe local stresses.

The fluid in which the dissection is first mounted usually becomes stained after a few months, as a result of blood pigment diffusing out of the specimen. In addition, a certain amount of debris may collect at the bottom of the container. Consequently, whenever practicable, a new dissection is left immersed in mounting fluid in its container, with the mouth of the latter covered with a sheet of glass to protect the contents from dust, for a period of from three to six months so that, when the fluid is changed, the inside of the container can also be cleaned out.

The container is sealed in the following way. The dissection is removed, and after the discoloured mounting fluid has been emptied out, the inside of the container is very thoroughly washed and dried. Then the specimen is replaced, and sufficient clean mounting fluid is added, so that the specimen is completely immersed.

Two $\frac{1}{16}$ inch diameter holes are drilled through opposite corners of the lid, which is cemented on with Tensol No. 3 cement, applied as described in Chapter 8. Throughout the period during which the cement is being polymerised, the inside of the container must be more or less continuously and effectively ventilated. The importance of ventilation is far greater during the

first half hour after the lid has been placed in position t[illegible] subsequently. Effective ventilation can be achieved by means of a low-po[illegible] electric vacuum pump, as shown on the right hand side of Figure 19. As t[illegible] mp sucks air

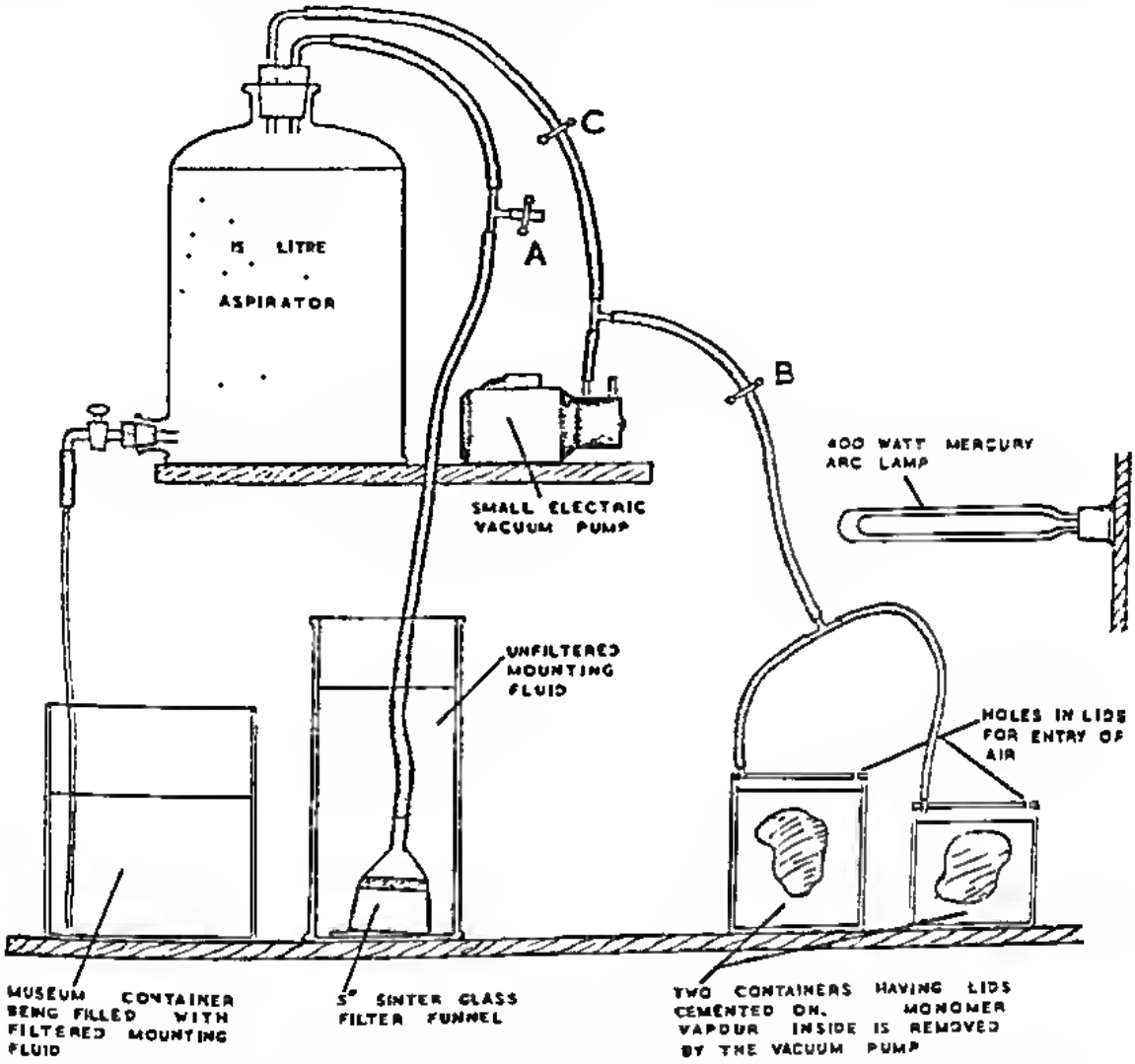

FIG 19

Diagram of the apparatus used to filter large quantities of mounting fluid, and also to remove monomer vapour from the inside of museum containers, while their lids are being stuck on A single low power electric vacuum pump can be used for both purposes, by means of the layout shown in the illustration When fluid is being filtered, clamps A and B are closed, and clamp C is opened. When filtered fluid is being run from the aspirator into a container, clamp A is opened, to allow air to replace the fluid running out of the aspirator When monomer vapour is being removed from the inside of containers, clamp C is closed, and B is opened

contaminated with monomer vapour from the cement, through one of the holes in the lid of the container, fresh air enters by the other.

The cement can be polymerised by the action either of sunlight, or by means of mercury arc lamps (see Chap. 8, and Fig. 16, p. 54). When the cement is quite hard, one of the holes in the lid of the container is sealed

by cementing a short length of $\frac{3}{16}$ inch diameter Perspex rod into it with Tensol No. 6 cement. To ensure a perfect seal, the rod is dipped in the cement to a depth equal to the thickness of the lid of the container, and held immersed in the cement for one minute. Then, after removal from the cement, the rod is pressed firmly into the hole, until its end is flush with the under surface of the lid. If necessary, the rod may be gently tapped into the hole with a light hammer.

The insertion of the rod is facilitated if its end is slightly bevelled before it is soaked in the cement. If by accident, a rod goes too far into the hole, it should be immediately removed and another piece of rod used. If the rod is left too far in, or partially withdrawn, a leaky joint is frequently produced.

Next the container is topped up with mounting fluid, until completely full. The remaining hole is then sealed in the same way. The rods are left undisturbed for at least two hours after insertion, while the cement hardens. Then the projecting ends are cut off with an old knife, the blade of which has been heated to dull redness. The hot blade passes through the Perspex, when pressed firmly against it, in the same way as an ordinary knife would sink into hard cheese. The knife leaves a smooth polished surface on the cut end of the rod.

The ropes and blocks supporting the sides of a large container can now be removed. The lid provides a considerable amount of support to the sides of the container. In addition to this, any tendency for the sides to bulge produces a partial vacuum inside, so that the sides are now partially supported by atmospheric pressure. In time the sides of a very large container become slightly convex, and the top slightly concave, but provided that sufficiently thick Perspex is used for the construction of the container, the distortion is never conspicuous. If it becomes necessary to change the mounting fluid of a very large container, the sides should first be supported with ropes and blocks, before a hole is drilled in the lid. If it is necessary to open a container, this is done after the fluid has been removed, by sawing off the top with the band saw.

Perspex containers should be polished periodically with Perspex polish No. 3 (see Appendix). This removes static electrical charges, produced when the containers are dusted, which attract particles of dust from the air, so that they stick on the surface of the container. The polish is effective for at least a month under normal conditions of humidity and temperature. Its effectiveness is not altered by dusting with a dry cloth, but is completely destroyed by washing.

Chapter 10

FIXATION AND MOUNTING OF VISCERA

IN order to obtain the most satisfactory results, certain viscera, destined to be displayed as more or less undissected specimens, require somewhat specialised treatment. In a pathology museum, where preservation of the original colour is more important than preservation of anatomical form, the specimen, either whole or sliced in half, is preserved by means of one of the modifications of the well known Kaiserling method of fixation. But in an anatomy museum, it is more important to preserve form than colour, and so the methods of fixing and mounting described below are adapted to this purpose.

The fixative recommended in certain cases would produce excessive hardening of the tissues, if the part was to be extensively dissected. But when the dissection, if any, is limited to such simple operations as cutting windows in the wall of intestine or heart, the hardening of the tissues is highly desirable, as it ensures that an otherwise somewhat flaccid part maintains its original shape when mounted.

Some viscera cannot be mounted satisfactorily simply by sewing them to a Perspex plate, owing to the curved surfaces of the specimen, and its texture, which would make the stitches liable to tear away. To avoid this tendency, these specimens are mounted by impaling them on a number of Perspex rods, previously cemented to the mounting plate. The organs dealt with below are the heart, stomach and gut, bladder, brain, liver, kidney and lungs.

The heart. When received from the post-mortem room the cavities of the heart usually contain gelatinous masses of coagulated blood. Before the heart is placed in fixative, the blood must be removed, as otherwise it later becomes very hard and clings to the walls of the cavities so tenaciously that it may be difficult to remove later without damage to the specimen. The blood is removed by inserting a glass cannula connected to the cold-water tap into the cavities, via the cut end of one of the vessels, and injecting water at fairly high pressure. If necessary the clots can be broken up by pressing on the walls of the heart with the fingers, while water is flowing through the cavities.

Next the cavities are loosely packed with wet cotton wool, introduced

with forceps via the cut ends of the vessels, to ensure that the walls of the heart are not fixed in a collapsed position. The heart is then placed in 10 per cent formalin for at least a week. This fixative, used in twice the concentration recommended for fixing material to be dissected, hardens the walls sufficiently to enable windows to be cut, even through the thin-walled atria, and for the cotton wool to be removed without the walls collapsing. If it is desired to fill the coronary arteries with a coloured mass, they must first be flushed with water and then 5 per cent formalin. These vessels can be filled either with gelatine (p. 20) latex (p. 22) or synthetic resin (p. 109). The heart is mounted in the usual way, by sewing it to a Perspex plate.

Stomach and gut. The contents are first washed out, and then one end of the specimen is ligated. The cavity is filled with 10 per cent formalin, until it is slightly distended, and then the other end is also ligated. The specimen is placed for at least a week in 10 per cent formalin supported by cotton wool, to prevent distortion which might result if the part rested directly on the bottom of the formalin tank. The fixing tank should be sufficiently large to permit the part to be fixed in such a way that the anatomical form is preserved. When the walls are sufficiently hardened, windows may be cut through them. The two ends of the specimen which were ligated are cut off to give it a neat appearance, and then it is mounted by sewing it on to a Perspex plate.

The bladder. It is desirable to show the bladder both with its walls contracted and distended. In the former case the part is fixed simply by placing it in 10 per cent formalin; in the latter the bladder is first distended by filling it with 10 per cent formalin. When sufficiently hardened, windows can be cut through the walls, and the specimen mounted by sewing it to a Perspex plate.

The brain. Care is needed to avoid distortion of the brain during fixation. Brains removed at post mortem are suspended by the proximal part of the basilar artery in 10 per cent formalin, in which the brain is totally immersed. The adjacent membranes are freed from the optic chiasma before the brain is suspended; otherwise the optic chiasma may be displaced posteriorly. The fixing fluid is renewed after four and fourteen days. After this period it is no longer necessary to suspend the brain, but it should be placed on cotton wool to avoid distortion due to it resting on the bottom of the fixing tank, and care must be taken to see that one brain does not rest upon another in the tank. To ensure complete hardening throughout the brain, it is left in the formalin for at least a further month.

Many workers have stated that it is necessary to inject fixative into the ventricles, to ensure that the central part of the brain is properly fixed. This is not only unnecessary, but a hazardous operation, for if the end of the injection needle happens to be in the brain substance instead of the ventricle during the injection, serious damage to the very soft substance of the unfixed brain may result. When the unfixed brain is lifted in the hands, the ventricles collapse, and the fluid in them is expelled. When the brain is subsequently suspended in formalin, the brain returns to its natural shape, and fixative flows into the ventricles. This process is repeated when the fixing fluid is changed, so that the central part of the brain is well bathed in fixative.

The most satisfactory way of fixing either a very thick slice, half a brain or a whole brain to the mounting plate is by impaling it on a number of Perspex rods, which have previously been cemented in position so that they project perpendicularly to the mounting plate.

Before a specimen is impaled holes must be drilled through it, slightly smaller in diameter than the diameter of the Perspex rods. The size of the holes must be such that the rods fit quite firmly, so that the specimen does not slide on them. But the rods must not fit so tightly that they cause the tissues around them to split. For all mounting by this method Perspex rods $\frac{3}{16}$ inch in diameter are suitable.

The only difficulty encountered in this work is to fix the rods to the mounting plate so that they are exactly opposite the holes in the specimen. In order to achieve this result, the holes are first cut in the specimen in the most suitable places. This is done by means of a straight piece of thin-walled brass tube, of external diameter equal to that of the Perspex rods. One end of the tube is sharpened on an oil stone, so that it resembles a cork borer (see Fig. 20). As the size of the hole cut is equal to the internal diameter of the tube, the diameter of the holes is slightly less than the diameter of the Perspex rods, which consequently fit firmly into the holes. In order to cut clean holes the boring tube must have a very sharp cutting end, and must be constantly rotated while it is being pressed through the specimen. Before the next hole is cut, the tissue in the borer is expelled by means of a ramrod.

When a sufficient number of holes have been cut, lengths of Perspex rod are inserted into each. The end of each rod is rounded before insertion so that there is no sharp rim to cut the tissues. But it should *not* be sharpened to a fine point, as this is liable to leave the hole so that the Perspex rod, which is quite flexible, pierces the tissues obliquely.

Next the specimen is placed on the mounting plate in exactly the position

it is to occupy in the museum container, with the Perspex rods perpendicular to its surface, and so adjusted that they just touch it.

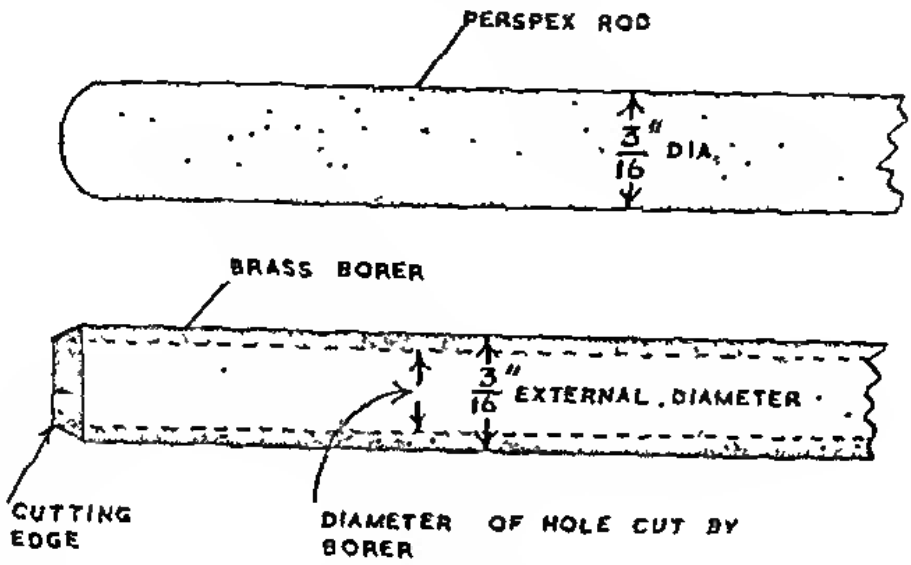

FIG. 20

Enlarged diagram of the end of a 3/16 inch diameter Perspex rod, and of the brass borer. Note that although the external diameter of the borer is 3/16 inch, the diameter of the hole cut is less, as it is equal to the internal diameter of the borer.

The places at which the rods touch the plate are marked with a China-graph pencil. Then $\frac{3}{16}$ inch holes are drilled to receive the rods. Suitable lengths of $\frac{3}{16}$ inch diameter Perspex rods are then cemented into each of the holes with Tensol No. 6 cement. This is done by dipping one end into the cement for one minute, and then pressing it into position. The rods are left undisturbed for at least two hours while the cement hardens before the specimen is impaled. It is advisable to leave lengths of Perspex rod in the holes in the specimen until the moment the latter is impaled, as otherwise in some cases the holes become partly closed as a result of the surrounding tissues swelling. After the specimen has been impaled the ends of the rods projecting beyond the specimen are cut off, by the method described on page 62. Brain slices of medium thickness, *i.e.* about 1 cm. and less, can be mounted more satisfactorily by sewing them to the mounting plate (see p. 231).

The liver. First the gall bladder is compressed to expel as much as possible of the bile. It is then filled with water and emptied several times, to remove all traces of bile, which stains the mounting fluid very badly. Next the hepatic artery is injected with a considerable quantity of cold deaerated water, which escapes via the hepatic veins, the portal vein, and to a lesser extent, via the bile ducts. This is done to wash out as much as possible of the blood and bile (see Fig. 2, p. 7, and text, p. 6 for injection method).

After injecting 5 per cent formalin into the hepatic artery, the vena cava is packed with cotton wool and the liver is left for a week in a tank of 5 per cent formalin. It is supported in the tank by cotton wool to prevent its surface becoming flattened.

Next the cotton wool is removed from the vena cava, which is sufficiently hardened to keep its shape without support. The liver is washed in running cold tap water for an hour and the gall bladder is flushed out with water, to remove the formalin from it. After compressing the gall bladder to remove as much fluid from it as possible, it is filled with a 15 per cent aqueous solution of gelatine, injected directly through its wall by means of a hypodermic syringe. The gelatine should be used only about three degrees above its setting temperature of 28°C, so that it sets almost immediately it is injected. It is not necessary to ligate the cystic duct, as the gelatine sets in this before much has escaped from it, provided that the gelatine is injected fairly slowly. If the gelatine sets in the needle of the syringe during use, it can be remelted instantly by pouring a little hot water over the needle. When the gall bladder is filled, the setting of the gelatine within it can be accelerated, and leakage through the puncture hole left by the needle prevented, by immersing the liver in very cold water. No advantage is gained by injecting coloured gelatine, as the walls of the gall bladder are too opaque to transmit colour.

It is desirable to fill with gelatine those parts of the hepatic artery and portal vein which are visible, so that they are held firmly in their normal position with the walls of the vessels round instead of being more or less collapsed. The filling with gelatine of a short length of a relatively large vessel like the portal vein, which has very flabby walls, so that the walls are firmly stretched, is a rather difficult operation, which requires much patience. This operation is complicated by the fact that there may be a number of leaks from the ends of small tributaries cut or even torn away from the main vessel at the time the liver was removed from the body. To do this work successfully two people are needed. The liver is placed in a fairly deep basin of ice water, and held in this so that the portal vein is above the level of the water. Twenty-five per cent gelatine solution which is on the point of setting is then injected into the portal vein from a hypodermic syringe, while the walls of the cut end of the vein are supported with forceps. The gelatine is injected slowly, and at the same time the liver is gradually lowered into the cold water. The gelatine which at first escapes from leaks soon sets and thus seals them. The needle is kept inserted some way within the vein and gelatine is allowed to overflow from the cut end and set. By injecting more gelatine after the

first deaerated, then fixed, and finally expanded by filling them with gelatine. though at the same time a comparatively large amount of gelatine may set outside the vein. When the gelatine has become quite firm, any outside the vessel is carefully dissected away by cutting it with a sharp scalpel. It is best to sew the common bile duct and hepatic artery to the portal vein, as the former are not rigid enough to be self-supporting, even when filled with gelatine, while the latter, because of its much greater diameter, is comparatively firm, especially when the gelatine has later been hardened in formalin.

After the fat has been trimmed away from around the vessels, and the remains of the diaphragm from the surface of the liver, all ragged parts are coated with gelatine and steamed by the method described in Chapter 7. Extreme caution is needed when steam is applied to the walls of the vessels

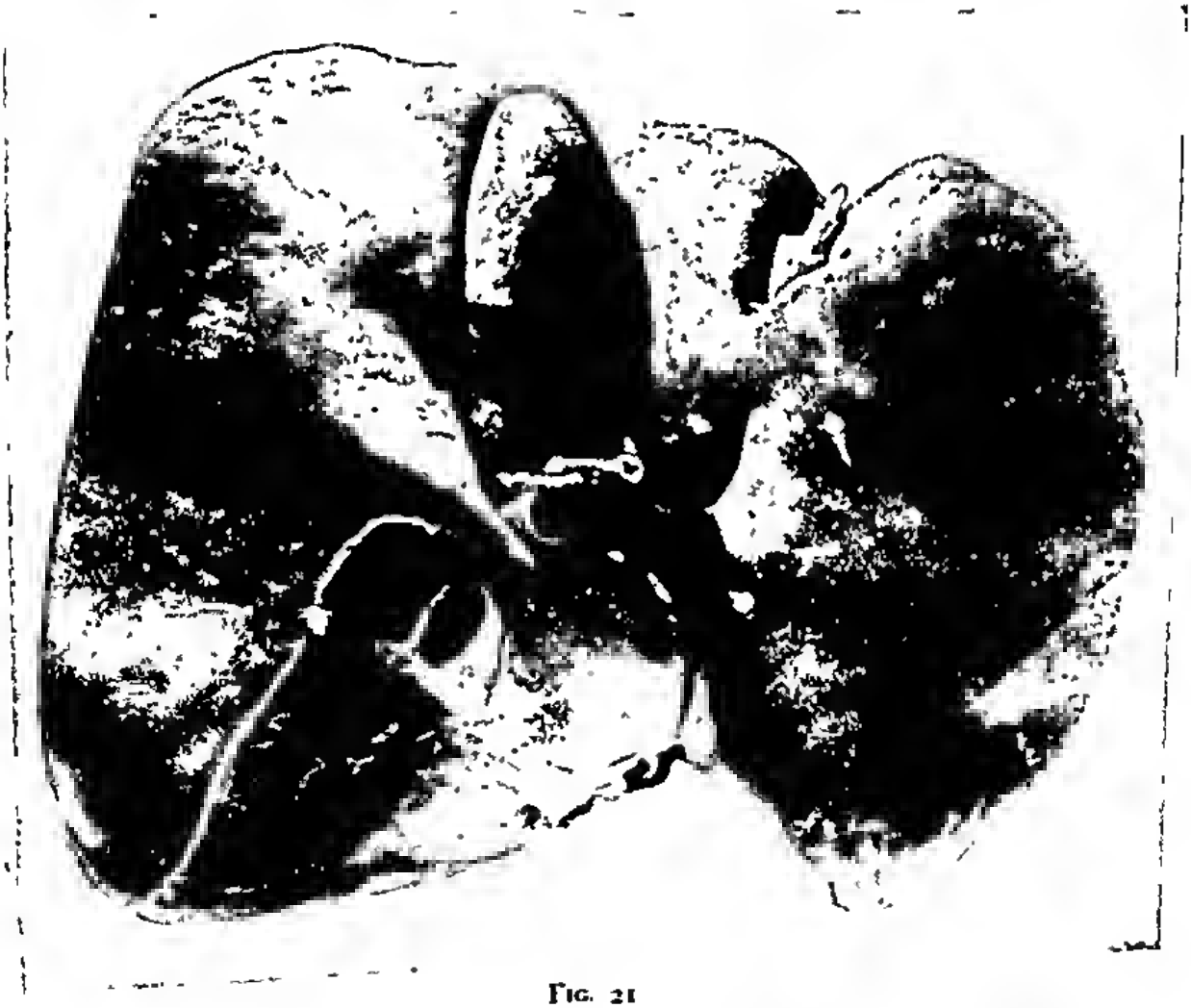

FIG. 21

Adult human liver, mounted as a museum specimen. The gall bladder and portal vein have been filled with gelatine to expand their walls. Note the ends of the rods by which the specimen is impaled.

filled with gelatine. A brief application of steam greatly improves the appearance of these vessels, but if it is applied for a fraction of a second too long, much of the gelatine within the portal vein melts and escapes and its walls collapse.

The most satisfactory position in which to mount the liver is with the tip of the gall bladder uppermost. The best way of fixing it to the mounting plate is by impaling it by the method described on page 65. Figure 21 shows a liver prepared and mounted by the method described above. As livers usually stain the mounting fluid very badly, the latter needs to be changed several times at comparatively frequent intervals after the specimen is first mounted. Two quite distinct types of discoloration occur. The mounting fluid may become green as a result of staining with bile; or it may become milky, as a result of other substances diffusing out of the liver. This milky appearance should not be confused with the milky colour which appears in mounting fluid, due to contamination of the latter with monomer from the cement used to fix the lid of the Perspex container on (see p. 59), although the two effects are very similar in appearance.

The kidney. The blood is washed out by injecting tap water into the renal artery. This is followed by 5 per cent formalin to fix the organ. The cut ends of the renal artery and vein are filled with gelatine by the technique described for filling the vessels of the liver, and a suitably curved glass rod is tied into the ureter to keep it in position. The specimen is cleaned and coated with gelatine by the method recommended for ordinary dissections. It is mounted by sewing it to a Perspex plate. The pelvis of the kidney is best displayed by slicing the kidney in half longitudinally before mounting, although very beautiful casts of this structure can also be prepared (see Fig. 76 p. 220).

The lungs. As received from the post-mortem room lungs are a shapeless mass. Before they are mounted, they must be fixed in the expanded condition, as nearly as possible in the form they had in the thoracic cavity during life. In order to achieve this, after the blood has been washed out, the lungs are first deaerated, then fixed, and finally expanded by filling them with gelatine.

A short length of Portex vinyl VY standard drainage tubing (see Appendix) is tied into the trachea to facilitate the connection of rubber tubing from apparatus. Portex tubing is preferred to glass, as the former is moderately flexible and, if tied firmly in place with fine string, it never slips out. This type of Portex tubing is used rather than Portex polythene tubing recommended elsewhere, as the latter is not made in sufficiently large sizes for this work.

The lungs are placed in the sink, which is filled with cold water in which they float. The cold tap is connected to the trachea, by means of rubber tubing, and water is run into the lungs via the trachea. The water escapes partly by diffusion through the pleura, but also via the pulmonary vessels, and so

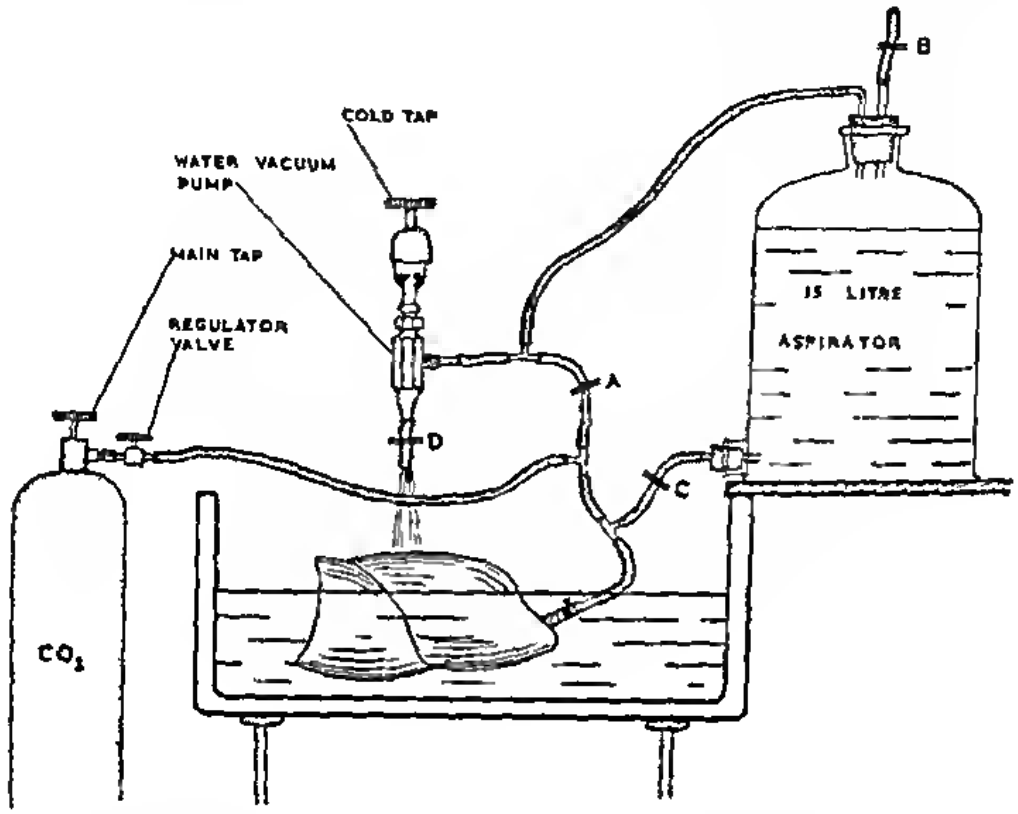

FIG 22

The apparatus for deaerating lungs. Screw clamps are fitted at A, B, C and D, by means of which the rubber tubing can be closed. By adjustment of these clamps the following operations can be carried out:

(i) Tap water can be run into the trachea, to wash out the bronchi and remove blood from the pulmonary vessels.
(ii) The lungs can be filled with carbon dioxide.
(iii) The lungs can be evacuated.
(iv) Tap water can be run into the aspirator.
(v) The water in the aspirator can be deaerated
(vi) The lungs can be filled with deaerated water.

washes out the blood from the latter. Washing is continued until the water escaping from the pulmonary veins is comparatively free from blood. It is advisable to remove most of the blood as this otherwise stains the mounting fluid badly.

Deaeration of the lungs is carried out with the apparatus illustrated in Figure 22. The lungs are alternately inflated with carbon dioxide until they are slightly over-expanded, and evacuated by means of the water vacuum pump a dozen times. This treatment results in practically all the residual air in the lungs being replaced by carbon dioxide. Next a large volume of

cold water, which has been previously deaerated in an aspirator by means of the water vacuum pump, is allowed to flow through the lungs. The water rapidly absorbs the carbon dioxide, so that the lungs now almost sink.

The lungs are fixed by running about 5 litres of 5 per cent formalin, made up in deaerated water, into them. Then they are placed in a tank of 5 per cent formalin for one or two days. They should not be left longer in formalin before they are filled with gelatine, as they collapse in the formalin, and the hardening of the tissues which results from more prolonged fixation may make it difficult to expand them fully again.

After removal from the formalin tank the lungs are placed in a sink filled with water at between 32°-35°C, and about 20 litres of deaerated water at this temperature are run into the trachea by gravity flow, to warm the lungs, and wash out most of the formalin, which otherwise might cause premature solidification of the gelatine, during the injection of the latter, as formalin renders gelatine quite insoluble.

Bone gelatine sheet, 120 bloom, which is supplied in the form of comparatively thick rectangular leaves, is recommended for filling the lungs. The gelatine is prepared for use in the following way. A sufficient number of leaves to make six litres of gelatine solution are laid out in cold water in large enamel meat trays, and left to soak for twenty-four hours. After this period they are saturated with water. It is important to arrange the gelatine leaves in a single layer while they are soaking as, if two leaves stick together, they may not be completely saturated with water after twenty-four hours. The soaked leaves weigh approximately nine times as much as the dry ones. This figure enables a fairly accurate estimate to be made as to how many leaves should be soaked. Gelatine solution prepared from leaves not fully saturated with water is unsatisfactory for this work, as it swells inside the lungs as a result of absorbing more water after setting, distorting their shape.

The soaked leaves are removed from the water and dried with a towel. They are now quite flexible, but firm (provided that this work is not attempted in very hot weather when the room temperature is approaching the melting point of the gelatine, which is 28°C). The leaves are packed into 7 lb. Kilner jars, which are placed in the sink, the latter being filled with hot water at about 50°C. The temperature of the water in the sink is maintained at about 50°C by allowing a slow flow of water into it from the hot tap. The gelatine is melted and heated to a temperature of 45°C. The lids of the jars should remain on (but not be screwed down) during this period, to prevent a skin forming on the surface of the melted gelatine. Thymol

crystals in a percentage of 0.1 are added to the gelatine while it is at 45°C. Although the gelatine is injected into the lungs at a considerably lower temperature, it is necessary to raise the temperature to 45°C to ensure that the thymol mixes thoroughly, as thymol is not readily soluble in aqueous solutions, and the crystals do not melt until they are heated to 41°C. The thymol is stirred thoroughly into the gelatine. It acts as a preservative, and prevents the possible growth of moulds in the gelatine, after the latter has been run into the lungs, during the comparatively long period which elapses before the formalin, in which the injected lungs are preserved, penetrates to the centre of such a solid mass of gelatine.

Before use the gelatine is cooled to 32°-35°C, by lowering the temperature of the water bath in which the jars are standing, and during this period the gelatine must be stirred vigorously and continuously, as otherwise gelatine cools and solidifies next to the glass, while remaining hot and liquid at the centre.

The gelatine is injected at a temperature of 32°-35°C. If used at a higher temperature than this it is too fluid, and diffuses too freely through the lungs, so that much of it escapes into the water in which the lungs rest; but if it is used at a temperature lower than 32°C, it is liable to set before the injection has been completed, especially if the room is rather cool.

Immediately before the gelatine is injected, the lungs are gently compressed with the hands, to expel as much as possible of the water within them. They are placed on a Perspex tray similar to that shown in Figure 32 (p. 135), which approximately resembles the posterior thoracic wall of a medium-sized individual, as this facilitates the arrangement of the injected lungs in the desired position, and avoids the risk of the surface of the injected lungs becoming flattened, as a result of their resting on the bottom of the sink. The method of constructing these trays is described on page 135, however it is possible to obtain satisfactory results by supporting the lungs with the hands while the gelatine sets, if a Perspex tray is not available.

The gelatine is run into the lungs from a large funnel, held about six inches above them by means of a clamp and retort stand. The funnel is connected by means of rubber tubing to the Portex cannula already tied into the trachea. The funnel is warmed by immersion in warm water immediately before use, to avoid the possibility of the gelatine setting in the apparatus before it starts to flow into the lungs.

When the lungs are full of gelatine, and consequently turgid, they tend to assume their natural expanded form, though they require a little adjustment

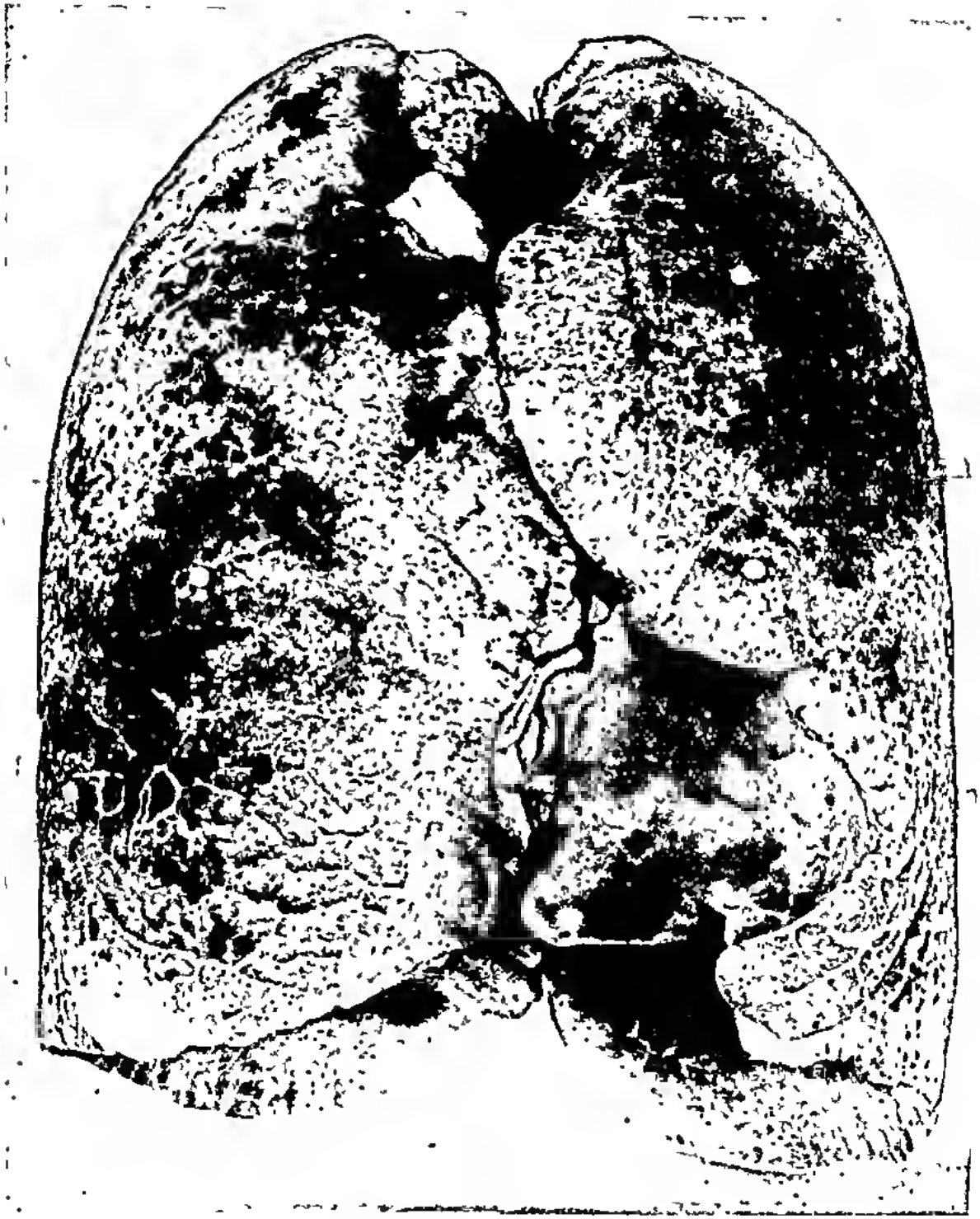

FIG 23

Adult human lungs, filled with gelatine to expand them and hold them in the anatomical position, mounted as a museum specimen Note the ends of the rods by which the specimen is transfixed and impaled

on the tray. The flow of gelatine is now checked by clamping the rubber tubing, and the water in which the lungs rest is cooled as rapidly as possible, either by running in water from the cold tap (in cold weather), or by adding a large quantity of ice. When the gelatine has set, the lungs are placed for a month in 5 per cent formalin made up in a 25 per cent aqueous solution of glycerine, to harden the gelatine, and ensure that the latter is completely impregnated with fluid identical with that used for mounting.

Before the lungs are mounted, the gelatine which has escaped from them and set on their surface is removed by scraping it away. After washing the hardened lungs in cold running water for an hour to remove formalin from their surface, the various structures such as aorta, oesophagus, and ends of the pulmonary vessels are trimmed, and coated with gelatine by the method used for finishing ordinary dissections (see p. 45). The specimen is then returned to the tank of preservative overnight, so that any water absorbed while the lungs were resting in water is removed by the dehydrating effect of the glycerine in the preservative.

Before the lungs are mounted two sharply pointed $\frac{3}{16}$ inch diameter Perspex rods are pushed transversely through both lungs to hold them in their correct position in relation to each other. The protruding ends of these rods are cut off by the method described on page 62.

The lungs are mounted by impaling them. They must be returned to the preserving fluid between the various manipulations. If they are placed in ordinary water they expand somewhat and their slightly altered shape may cause serious difficulty in the impaling technique.

The weight of the injected lungs is such that the container in which they are mounted should not be stood upright until at least half full of mounting fluid, which then supports much of their weight. Figure 23 shows human lungs, prepared and mounted by the method described above.

PART II

THE ILLUSTRATION OF ANATOMICAL DISSECTIONS

Chapter 11

INTRODUCTION

IN a teaching museum each anatomical specimen should be accompanied by a verbal description. In many cases the value of the dissection is increased if an illustration is also provided, as this facilitates the verbal description and makes it easier for students to identify the various parts. It also provides a means of drawing attention to inconspicuous but important structures, which might otherwise be overlooked.

Three types of illustration may be provided, consisting of either photographs, outline drawings, or anatomical illustrations in which each structure is carefully portrayed. Whichever method is used extensive labelling is necessary.

Photographic illustrations (see Fig. 24, p. 86) are the easiest to produce, but for several reasons they are usually unsatisfactory. The camera does not discriminate, so that important features are often masked by unimportant details. If the lighting is arranged to throw some areas of the dissection into sharp relief, others appear very flat, or may even be obscured by shadows. When the dissection is viewed, the complete course of some structures such as arteries and nerves can be seen only if the head is moved slightly, so that in a photograph these are partly obscured. Although the value of photographs can be increased by skilful retouching, they must nevertheless be regarded as the least satisfactory form of illustration for anatomical dissections.

Outline or key drawings (see Fig. 25, p. 87) can be made fairly easily, by first tracing the outline of the principal structures from a photograph of the specimen, and then adding by free-hand a simplified outline of any smaller structures to which it is necessary to draw attention. A key drawing is easier to interpret if the arteries, veins and nerves are coloured. This type of illustration is more satisfactory than a photograph, but the identification of the numerous labelled outlines on the actual dissection may require a considerable amount of effort. This may have the advantage of fixing more firmly in the student's mind the relationships concerned, but outline drawings are aesthetically unsatisfying, and almost useless when viewed apart from the dissection, so that they cannot be used to illustrate articles or text books.

Anatomical drawings (see Fig. 26, p. 89) in which each structure is

carefully portrayed, take much longer to make than simple outlines; but when it is recalled how long an elaborate museum dissection takes to complete, and in view of the fact that these dissections may be expected to last considerably more than a hundred years, the extra time required to make really attractive drawings is not difficult to justify.

When an anatomical drawing is made, the important features are accentuated, and unimportant details omitted or subdued. Consequently a satisfactory anatomical drawing can be made only by someone who understands the dissection. There are a limited number of professional artists whose knowledge of anatomy is sufficient to enable them to undertake this work successfully under the supervision of an expert anatomist; but more often than not, the only person available to make the drawings is the prosector who made the dissection. Although his training does not usually include a course at an art school, this is not an insuperable obstacle to the production of satisfactory anatomical drawings. Anyone who has the ability to master the technique, not formally taught in any school, of making first-class museum dissections, is also capable of mastering one of the techniques by which anatomical drawings can be made.

Although it would be idle to pretend that lack of art training is not a handicap to the potential anatomical artist, there are nevertheless some actual advantages attached to this. In normal art training, the student is taught to portray accurately what he sees, but the anatomical artist never does this. He selects, simplifies, and reduces to a semi-diagrammatic style, so that an artist unversed in the principles of anatomical drawing would be astonished by the appearance of the completed illustration. While the ordinary artist is taught to avoid putting too much detail into his work, as for example the pitfall of drawing every brick in a wall, and is encouraged to suggest the actual details by means of a few lines, the anatomical artist must draw exactly and clearly all the features which he has selected as being sufficiently important to illustrate.

Chapter 12

MATERIALS AND METHODS

A GREAT many beautiful anatomical illustrations have been produced by means of the Ross Board and Air Graph techniques. But as both of these techniques are highly specialised and make great demands on the skill of the artist, they cannot be described here. They should be learnt at a school of art, or under the instruction of a qualified medical artist. Only comparatively simple techniques, which can be mastered by those with aptitude, even if they have had little formal art training, come within the scope of this book.

The three techniques most frequently used for making anatomical drawings are :

1. Line drawings made entirely with pen and ink.
2. Smudge drawings, made with some kind of soft pencil, the marks of which can be smudged to produce soft tones of light and shade.
3. Wash drawings in which water colours are applied by means of brushes.

Each artist should select the method by which he can obtain the best results. Some may excel at all three types of technique, but a less versatile person may have to be content with perfecting one. Many artists combine all three in the same drawing.

Line drawings are usually made with black waterproof ink, applied with a fine pen to Bristol board or scraper board. Bristol board is the easier of the two to use, as its very smooth surface facilitates the drawing of fine and even lines. Errors can be erased by scraping the surface with a sharp scalpel. Scraper board consists of a cardboard faced with a smooth surface of chalk. When drawing with ink on scraper board, the nib must be frequently cleaned, as it is quickly clogged by particles of chalk picked up from the surface of the board. The advantages of scraper board are the facility with which alterations can be made to the drawing by scraping away the incorrect part with the blade of a scalpel, and the fact that white lines can be made by covering an area with ink, and then scraping the surface, when the ink has dried, with various tools designed for this work. If the original shading of

with a wad of cotton wool. Care is also needed to avoid smudging the part of the drawing already completed, while working on another area. The completed drawing must be sprayed with fixative to prevent further smudging when it is handled. After fixation, high lights may be added by applying Process white with a sable brush.

Wash drawings can be made on a variety of surfaces. Whatman's hotpressed watercolour board (see Appendix) is however particularly recommended, as this is one of the easiest surfaces on which to work. It consists of a fairly smooth paper, stuck to a stout cardboard backing, which prevents the paper wrinkling when it becomes wet. The drawing may be made with any dark pigment, but it is important to select one which is permanent, *i.e.* does not fade. For general work neutral tint is recommended. This appears almost black when applied in concentrated form, but when diluted with water, gradually lightens to a pleasing bluish grey.

A selection of sable water-colour brushes ranging in size from No. 00 to No. 3 are required. It is essential that the brushes shall be of the highest quality. This is indicated by the springy nature of the sable hair, and by the very fine but firm point produced when the brush is wet. Windsor and Newton's Series 7 brushes are highly recommended.

Although a wash drawing takes longer to complete than the same illustration would take if made in line or smudge, it has the advantage that both fine detail, and large areas of half tone can be produced with equal ease. Finer lines can be more accurately drawn with a No. 00 brush than with the finest pen, provided that the brush is used in the correct way. To draw really fine lines, the brush must be loaded with just the right amount of paint, and *the hairs pressed together by rolling the point in the palette.* When making fine lines, the point of the brush must only just touch the paper, and the handle must slope in the direction of the intended line. The line should be drawn away from the body, even if this involves inverting the drawing. Half tones are applied by first covering the area with a faint even wash by means of a fairly large brush well loaded with fluid. When the first coat is almost dry, a second is applied to those parts it is desired to darken. Successive coats of slightly darker paint are applied where required, but care is needed to prevent the paper becoming waterlogged, as then it no longer holds the paint. On the other hand, the paper must be kept slightly damp to avoid brush marks. The latter can be almost completely avoided by skilful work, but if they are present in the completed drawing they can be removed in three ways; either by gently rubbing the darkest part of the brush mark with a clean damp brush,

any area by means of lines is too dark, this can be lightened by scraping white lines over the black ones, or by scraping each black line to make it thinner.

The principal drawback to the use of a line technique is the difficulty of representing smooth curved surfaces, as the drawing is composed entirely of lines and dots. Every effect has to be produced by variations of the thickness, direction, length and proximity of the lines, and the size, shape and proximity of the dots. This drawback can be overcome by making only the outline of the drawing in line, and filling in the half tones with smudge or wash. But if this is done, the line drawing must be made on a board or paper which is suitable for the particular half tone technique employed. Neither Bristol board nor scraper board is suitable for smudge or wash techniques.

Smudge techniques are usually applied by using either various grades of graphite pencil on a smooth hotpressed cartridge paper, or Wolff's carbon pencils (see Appendix), or Conté drawing pencils (made in France), on a rougher-surfaced paper. Conté pencils produce particularly black tones. Fine lines are drawn with the harder pencils, and the tones applied by first lightly passing the side of the blunt point of a very soft pencil across the paper so that it makes comparatively light but broad marks, and then rubbing the marks, either with special stubs, a small piece of cork in a holder, or a little pad made by tying up a small ball of cotton wool in a piece of chamois leather. The smooth tones produced in this way are gradually intensified by repeating the process. The drawing can be lightened by means of a rubber. Putty rubber is particularly suitable for this, as it can be squeezed into any shape, and its surface comes away very freely when it is used, so that a clean surface of rubber is always in contact with the drawing. Weldon Robert's Graypoint eraser No. 365 (made in U.S.A.), consisting of a hard rubber enclosed in wood like the lead of a pencil, is also very useful at times.

Smudge drawings can be made on many different surfaces, but the surface chosen must be suited to the type of pencil used. Generally speaking, when a pencil is used which leaves marks composed of very small particles, a comparatively smooth-surfaced paper should be used; but with a coarse-grained pencil a rough-surfaced paper is best.

When a smudge technique is being used, the greatest care must be taken to avoid getting any grease on the surface of the paper. If the surface to be drawn on is touched with the fingers, fingerprints may appear when the half tones are applied. However, grease can be removed with benzene, applied

with a wad of cotton wool. Care is also needed to avoid smudging the part of the drawing already completed, while working on another area. The completed drawing must be sprayed with fixative to prevent further smudging when it is handled. After fixation, high lights may be added by applying Process white with a sable brush.

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which removes a little of the paint; or by delicate cross hatching applied to the lightest part of the brush mark with a No. 00 brush charged with a small amount of paint of the required tone; or by gently rubbing the darkest part with a hard india rubber, after the paint is perfectly dry.

When water-colour drawings are being made, the greatest care must be taken to avoid the necessity for alterations after the wash has been applied, as it is difficult to apply wash smoothly to a surface roughened by erasion. However, if alterations are necessary, the paint can be removed from an area by persistent gentle rubbing in different directions with a hard rubber. It is sometimes necessary to mix Process white with the neutral tint, to give the latter more body, when painting over such roughened surfaces. As in the case of smudge work, care must be taken to avoid getting grease from the fingers on to the surface of the paper to be used for a wash drawing, as this makes the application of even coats of paint impossible.

Whichever technique is selected for making the drawing, the initial procedure is the same. First a photograph is taken of the dissection from a sufficient distance to avoid excessive distortion. An enlargement is made of suitable size, which forms the basis of the drawing. It is advisable to make the drawings relatively large at first as, although this may increase the time it takes to complete the illustration, it also reduces the technical difficulties.

Next a tracing of the outline and more conspicuous features is transferred to the paper or board on which the drawing is to be made. The general appearance of the finished drawing is greatly improved if the outline is corrected to remove any unintentional asymmetry in a bilateral dissection, and any post mortem distortion of structures. Next those anatomical details which it is desired to portray are carefully drawn in, with any modifications which will make the illustration clearer, without upsetting the general proportions of the different structures, and the anatomical relationships. When this stage has been reached, it is important that the drawing be very carefully checked, to observe if any inaccuracies of anatomical significance have been introduced during the previous work, as alterations at a later stage may spoil the appearance of the finished drawing.

After this stage has been reached, the subsequent work depends on the technique employed and the particular style developed by the individual artist.

If the completed drawing takes up too much room in the museum, a photographic copy, reduced to a convenient size, can easily be made without loss of detail. The best results in the case of half tone drawings are obtained by making contact prints from relatively large negatives, say 10 x 8 inches, on soft gradation panchromatic plates. The negative must be of just the right density to preserve the full range of half tones. If the drawing is to be reproduced photographically, only very light colours should be used to tint the arteries, veins and nerves, so that these structures will be reproduced in a light tone. They can then be brightly coloured in the photographic print by means of transparent photographic tints.

Chapter 14

A TECHNIQUE FOR THE PREPARATION OF HALF TONE ILLUSTRATIONS

AS the present writer neither enjoys the advantages, nor suffers from the handicaps, of art school training, it may be of interest to those in a similar position to know the details of his own personal technique.

The drawings are made on Whatman's hotpressed water colour drawing board (see Appendix). The half Imperial size measuring 15 x 12½ inches is large enough for all drawings, and can be cut smaller as required. Speera magnifying spectacles (see Fig. 11, p. 35) giving magnification of 1.75 diameters are recommended for all fine work, as these eliminate eye strain.

When the pencil outline has been completed and checked, the outline of the principal structures is drawn in with a Gillot's 659 Crowquill pen, using Windsor and Newton's Mandarin black waterproof carbon ink which has been diluted by the addition of 50 per cent distilled water. This enables an outline composed of very fine black lines to be made. The principal bundles of muscle fibres, lobulations of glands and fat are then drawn in with ink which has been diluted until it produces only faint grey lines.

Next all traces of the original pencil lines are removed with a paper-cleaning rubber, leaving a perfectly clean outline of the drawing, which includes all essential details. The arteries, veins and nerves are now tinted lightly with water-colour paints in the conventional colours, as it is found that this makes the subsequent work easier to do satisfactorily. Figure 24 shows a photograph of part of a dissection, and Figure 25 the completed outline drawing traced and modified from this photograph.

The background to the vessels and nerves is now filled in with neutral tint, applied with sable brushes ranging in size from No. 00 to No. 3. All the detailed work is done with the finer brushes, No. 00 to No. 1. The darkest parts are first covered with a very concentrated paint, so that they are practically black. Then the rest of the detail is filled in area by area, one area being completed except for the final shading, before the next is commenced. It is found that the area first completed provides a guide which makes the subsequent work much easier. But it is better to err by making the whole drawing a little too light than too dark, as it is much easier to darken

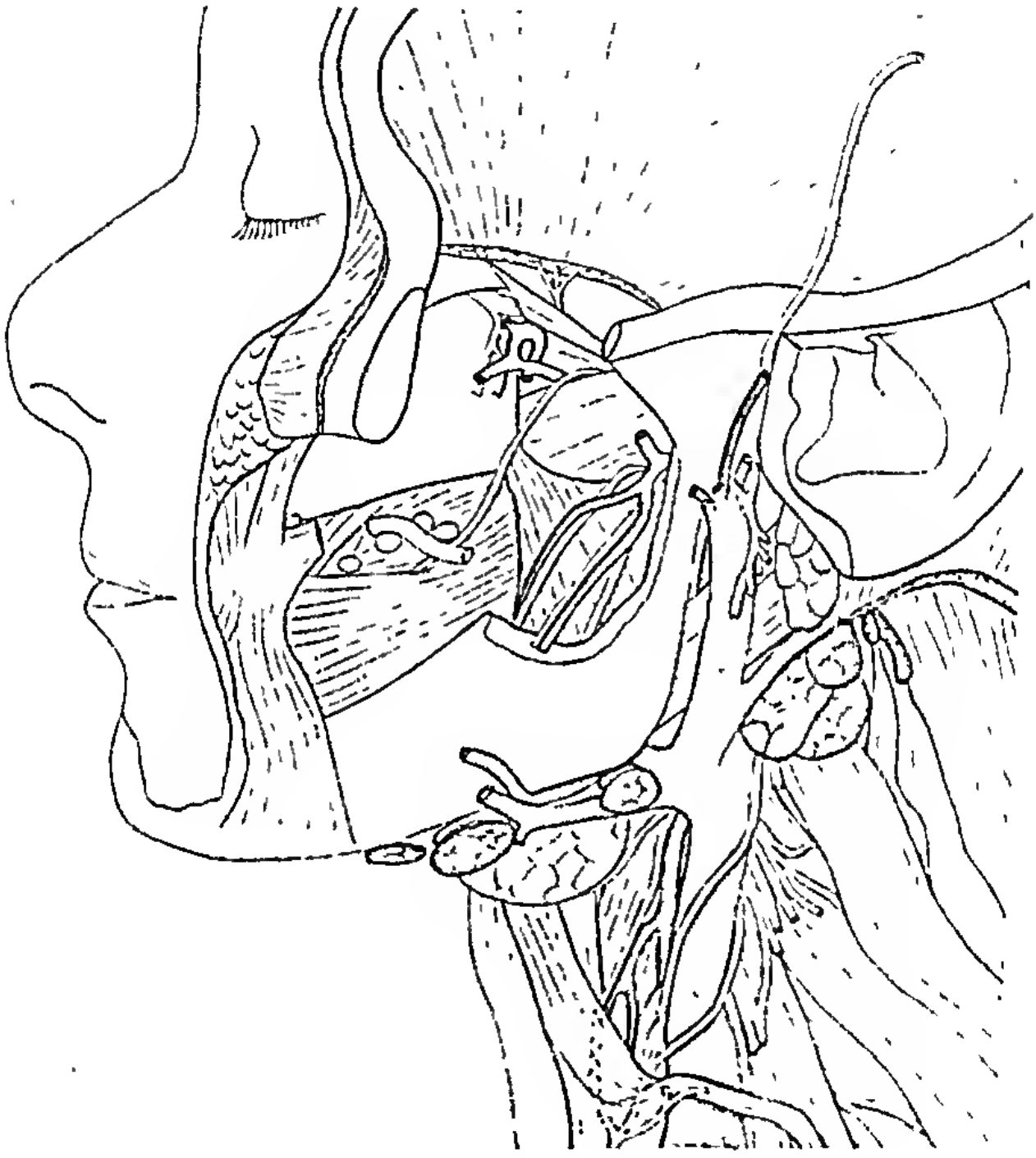

Fig. 25

Outline drawing based on the photograph in Fig. 24. This drawing represents the half way stage in the production of the half tone anatomical drawing shown in Fig. 26.

FIG. 24

Photograph of part of a museum dissection, used as a base for an anatomical drawing. × 3/4

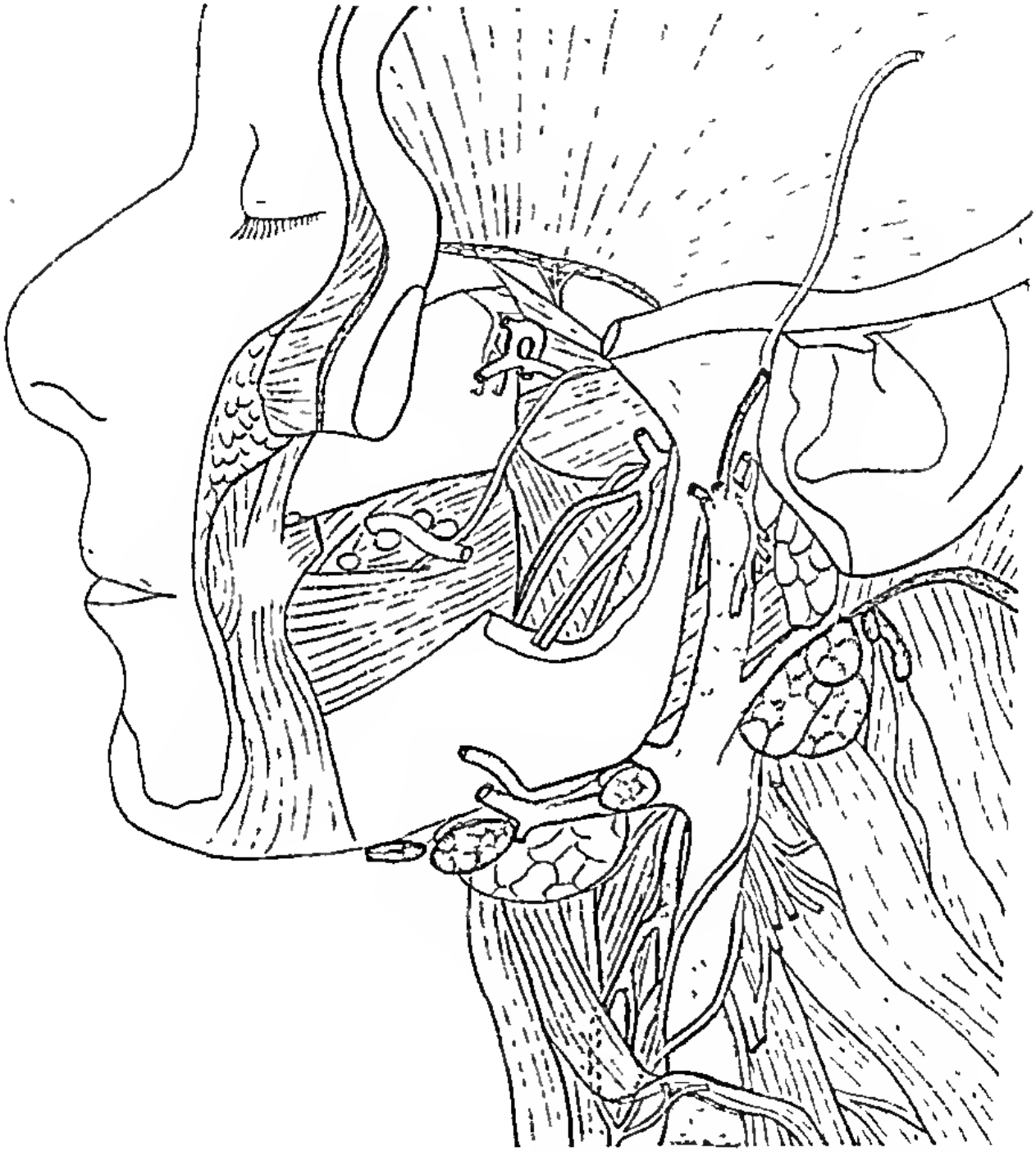

FIG 25

Outline drawing based on the photograph in Fig 24 This drawing represents the half way stage in the production of the half tone anatomical drawing shown in Fig 26 × 2/3

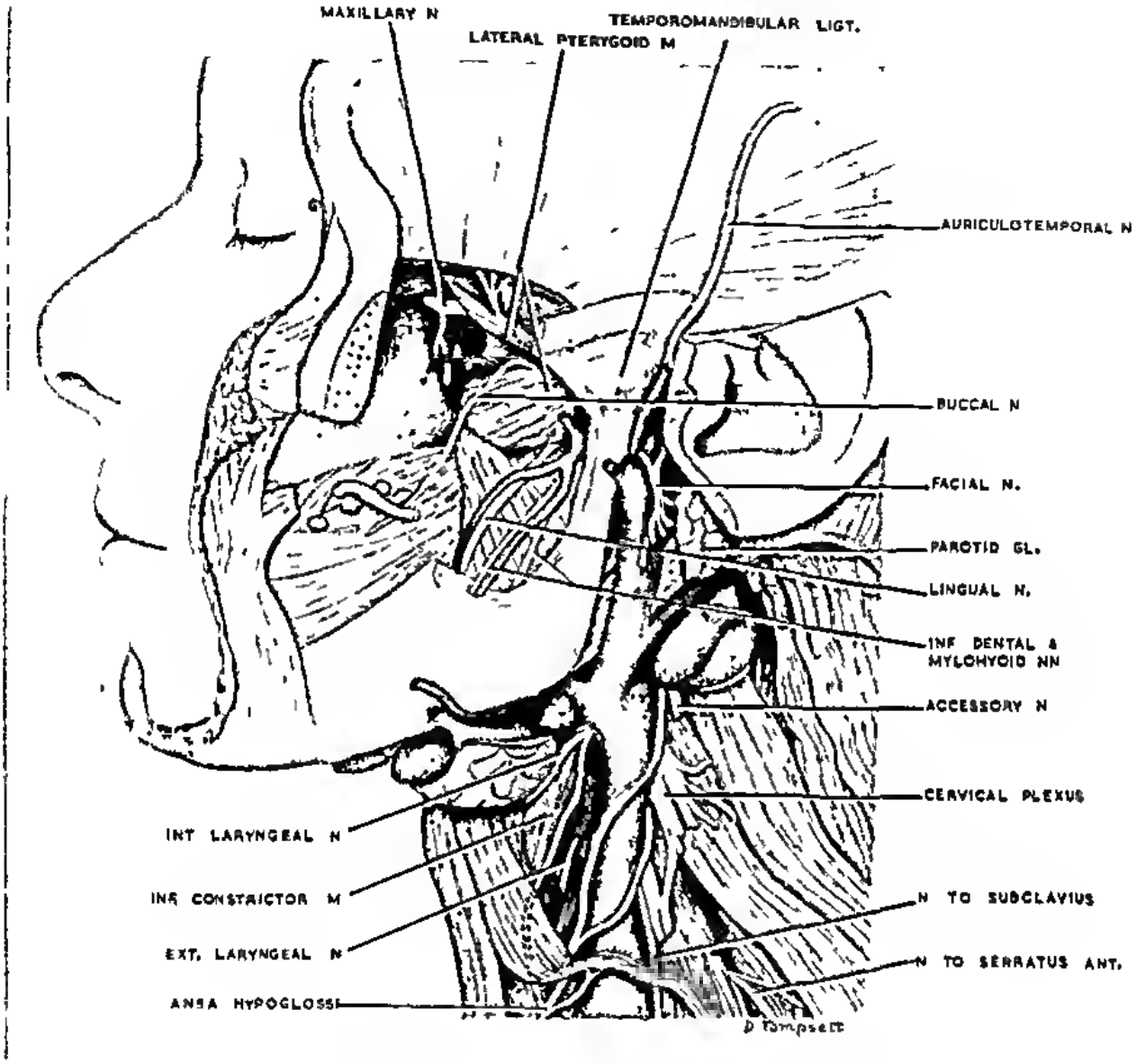

FIG. 26

Completed half tone anatomical drawing of part of a museum dissection (compare with Figs 24 and 25) The original drawing was made twice linear this size

must be removed by touching the neck of the bottle with the nib, so that excess of white runs back into the bottle. Failure to take this precaution may result in a blot of white on the drawing.

Although it is a little difficult to use, it is well worth mastering the technique of reinforcing each black line with white, as this not only makes the lines stand out much more clearly, but also makes their presence interfere less with the view of the drawing. Finally the labels are stencilled in.

Figures 24, 25 and 26 show respectively, a photograph of part of a museum dissection, the inked-in outline of a drawing, with the vessels and nerves tinted with colours, and a reproduction of a completed drawing, made by the particular technique employed by the writer. The original drawing was made twice linear the size of this reproduction.

If required for illustrating an article or a book, anatomical drawings made in line, smudge or wash are all suitable for reproduction by means of blocks. Line drawings in black and white are by far the cheapest to reproduce, and so should be used whenever they are adequate. Colours should only be used when absolutely necessary to produce a clear illustration, as they add greatly to the cost of reproduction.

PART III

CASTING IN SYNTHETIC RESIN

Chapter 15

INTRODUCTION

THE earliest recorded example of casts being made of an anatomical cavity are those of the cerebral ventricles by Leonardo da Vinci (1452-1519). Leonardo da Vinci used molten wax for this work, and it is interesting to note that he understood the physical problems involved sufficiently clearly to provide an escape hole for the fluid or air displaced by the wax. But as he had no method of hardening the brain before making the injection, the casts obtained bore little resemblance to the actual cavities.

The introduction by Jan Swammerdam (1637-1680) of molten wax injection masses for filling arteries, veins and ducts, pointed the way to the preparation of corrosion casts, in which, after the injection mass has solidified, the original tissues are corroded away by means of some reagent which does not attack the cast.

Although wax masses have the advantages that they can be brilliantly coloured and are completely resistant to concentrated hydrochloric acid, which can therefore be used to dissolve the organic tissues, the casts produced are very fragile. It was therefore inevitable that the quest should continue for more suitable materials for making corrosion casts.

The next substance to be used for this work was a low melting point metal alloy, with which Govert Bidloo (1649-1713) succeeded in making casts of the bronchial tree. Although metal casts are stronger than wax ones, there are two serious drawbacks to the general use of metal for this work. It cannot be coloured before the injection is made to differentiate between the various structures, and its weight tends to distort thin-walled cavities filled with it.

However, in spite of these drawbacks, until the end of the nineteenth century low-melting-point metals were the most suitable materials available for making corrosion casts, and this work was facilitated by the discovery of alloys of increasingly low working temperature. Outstanding among the casts made with metal were those of the cerebral ventricles made by Gustav Retzius (1842-1919). Some of the illustrations of these casts are still used in standard text books of anatomy; but Retzius himself emphasises the difficulty of producing a complete cast of the cerebral ventricles in metal, and states that

larger vessels is required, the viscosity of the injection mass is increased, so that it solidifies before reaching the very fine vessels.

In 1936 Narat and others described the use of a vinyl resin, known commercially as Vinylite, dissolved in acetone, as a substitute for cellulose acetate. Vinylite casts are superior to celluloid ones, and this material has replaced celluloid and been extensively used for making corrosion casts, particularly of the ducts and vessels of the liver, and of the respiratory tree and vessels of the lungs. The work of Liebow and others (1947) on the lungs deserves special mention, as the practical details of a somewhat complicated technique are very clearly and completely described.

The appearance in 1948 in England, and somewhat earlier in the U.S.A., of cold-setting synthetic unsaturated polyester resins, sold under such trade names as Marco resin and Castolite, opened up completely new possibilities in the sphere of anatomical casting. They make it possible to produce beautiful, rigid, coloured casts of any comparatively large anatomical cavity. The shrinkage of the resin during setting is negligible and, when fully hardened, it is strong and does not warp. Synthetic resin casts are reputed to last indefinitely without deterioration. These resins may also be used for making transparent casts and for embedding delicate coloured casts in rectangular transparent blocks of resin for protection. They are not generally suitable for filling very small cavities such as blood vessels of internal diameter less than one millimetre as, unless special precautions are taken, they do not set hard in the latter, the setting being inhibited by the water in the walls of the vessels.

For all the casting techniques described below, Marco resin 26 C (see Appendix) was used. The majority of them are corrosion casting techniques, although in one case the original tissue (the brain) is dissected away instead of being dissolved. Other techniques included involve the construction of wax models, the construction of negative plaster moulds, and of reproducing an original wax model in resin.

The one great drawback to the use of synthetic resins for making corrosion casts of the cavities of blood vessels and ducts is the impossibility of controlling exactly the extent of the injection. Consequently extensive pruning is necessary, as the cast of the fine branches obscures the view of the larger structures. In some cases the pruning is a very laborious task. However, the simplicity of the apparatus required, the consistently excellent results obtained if these techniques are skilfully applied, and the permanency of the casts, provide ample compensation for this labour.

Chapter 16

THE PROPERTIES OF THE RESIN AND ACCESSORIES

MARCO resin 26 C, manufactured by Scott Bader & Co. Ltd., has been used exclusively for all the casting techniques described below. This resin consists of a pale yellow transparent syrup, indistinguishable in appearance from golden syrup. The manufacturers have succeeded in making samples of this resin almost water white, but so far the cost of refinement has been too great for water-white resin to be produced commercially.

Between 10 and 40 per cent of a mobile colourless liquid, called monomer C, must be added to the resin before use, the amount used depending on the required viscosity.

Polymerisation of the resin mixture is stimulated by adding catalyst H C H and accelerator E. For normal applications not more than 5 per cent of each of these reagents should be used. The catalyst is a white powder. It is dissolved in the monomer, which is warmed gently over a water bath, as the catalyst does not dissolve readily in cold monomer. But the monomer must not be heated to a higher temperature than 40°C. After the catalyst has been dissolved in the monomer, the latter is incorporated in the resin.

Immediately before use the accelerator E, which consists of a mobile violet liquid, is added to the resin mixture and very thoroughly stirred in. After the accelerator has been added the resin mixture darkens slightly. Then its viscosity gradually increases until the mixture gels. The period of time which elapses after the accelerator has been added until the resin gels is referred to as the working life of the mixture. Mixtures suitable for various applications can be prepared in which the working life varies from ten minutes to several days, by adjustment of the catalyst and/or accelerator content. Just before the resin gels, its colour becomes noticeably lighter, and this colour change in certain circumstances gives a valuable warning that the resin is about to gel. In the case of a mixture with a working life of more than an hour, there is little change in temperature until the resin is on the point of gelling. But as the working life of the mixture is shortened by increasing the catalyst and or accelerator content, the rise in temperature which takes place before the resin gels increases progressively (see Fig. 28).

weight of resin, 20 parts by weight of monomer C, 4 parts by weight of catalyst H C H, and 4 parts by volume of accelerator E, at three different room temperatures (the composition of these resin mixtures can be conveniently written in the order above without actually naming the ingredients thus (100 : 20 : 4 : 4)). It will be seen from these graphs that within the range of 15°C, to 25°C, an increase of 5°C in the working temperature almost halves the working life of the resin. In addition the graphs show that the rise in the temperature of the resin which takes place before it gels increases as the working temperature increases.

In order to get the best results in many techniques, it is necessary to use resin mixtures which have a working life of about fifteen minutes, but which do not heat up to a temperature greater than about 30°C before they gel. This is impossible to arrange if the room temperature is much above 20°C and therefore, if this work is undertaken in tropical countries, an air-conditioned laboratory is essential.

No matter at what room temperature this work is done, a mixture with a working life of much less than fifteen minutes always heats up excessively before gelling, as shown in graph A in Figure 28, which represents the behaviour of a resin mixture containing an unusually large amount of catalyst and accelerator, polymerised at the normal room temperature of 20°C.

It is advisable not to use these resin mixtures when the room temperature is below 18°C, owing to the increasingly noticeable and somewhat unpredictable effect a lower temperature has on the working life of the resin.

If the resin sets in contact with water, the latter has a noticeable retarding effect on the rate of polymerisation. The water temperature must be raised about 5°C to compensate for this effect.

It is convenient to make up the resin mixtures in units containing 100 g. resin. When the other ingredients have been added, this produces roughly 100 ml. of resin mixture. Although resin, monomer and catalyst can be measured conveniently by weight, the accelerator is measured by volume, by means of glass pipettes, as in this way the desired quantity can be measured accurately and quickly.

A rough estimate of the working life of any uncoloured resin mixture can be made in the following way. At a room temperature of 20°C the mixture (100 : 20 : 4 : 4), has a working life of about 15 minutes. If either the catalyst or accelerator content is halved, the working life is approximately doubled. Thus the working life of the mixture (100 : 20 : 4 : 2) or (100 : 20 : 2 : 4) is 30 minutes while that of (100 : 20 : 1 : 1) is four

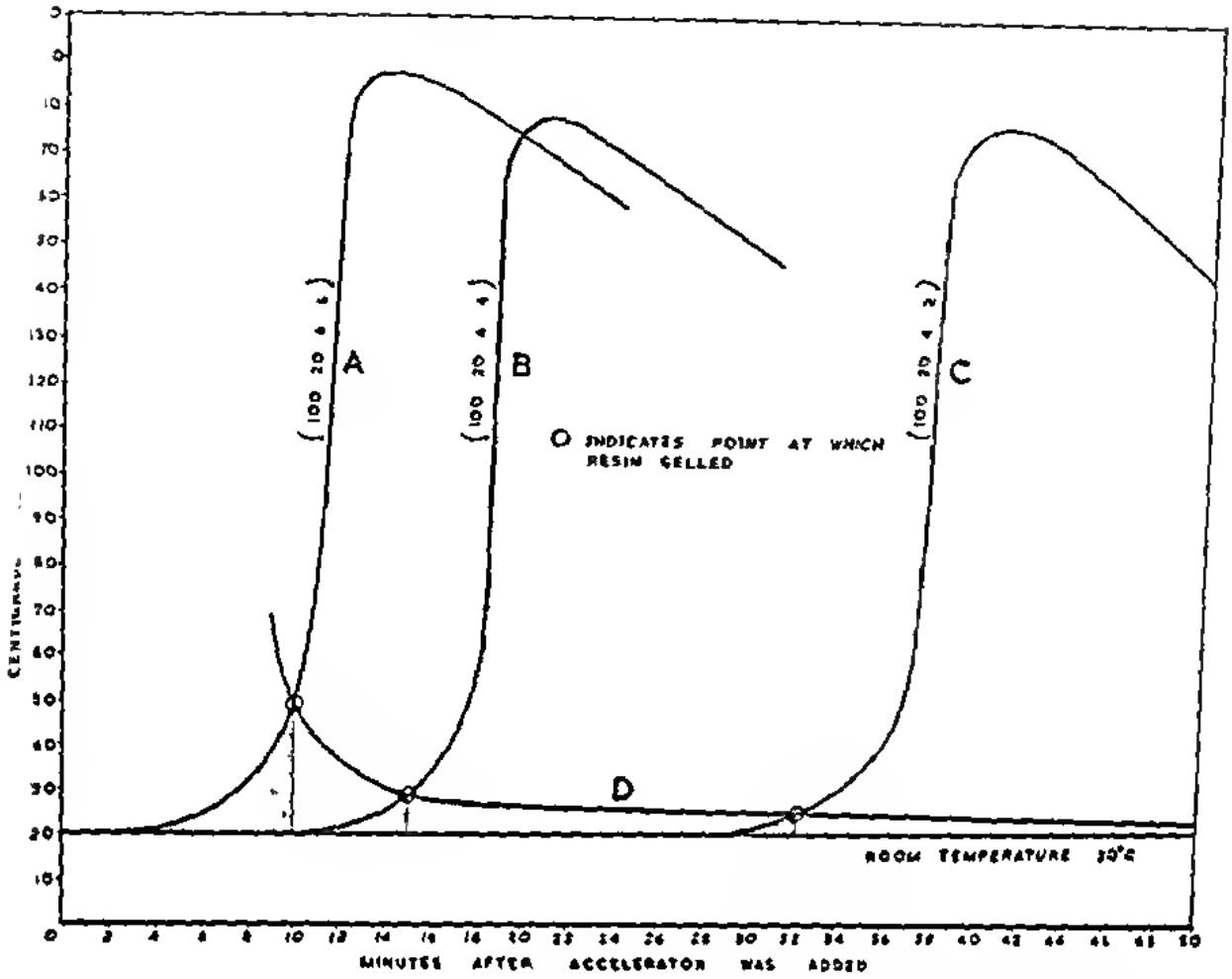

FIG 28

Graphs A, B and C show the rise in temperature which takes place during polymerisation in three different Marco resin 26 C mixtures, the composition of which is indicated by the figures in brackets beside each curve. The figures refer to resin, monomer, catalyst H C H and accelerator E respectively, the first three being measured in gms, the last in ml. In each case 50 ml were allowed to polymerise in a 50 ml. beaker, standing in air. Stippling draws attention to the rise in temperature which occurred in each case, before the mixture gelled. Graph D shows the relationship between the working life of the mixture and the rise in temperature which occurs before gelling commences.

hours. The monomer content of the mixture does not noticeably effect the working life.

Immediately after the resin mixture has gelled, or just before gelling takes place in mixtures with a high catalyst and accelerator content (see Fig. 28) there is an increasingly rapid evolution of heat, so that if a large mass of resin is involved, and no special steps are taken to cool it, the temperature may rise to over 200°C. The maximum temperature reached after gelling is not affected much by the catalyst and accelerator content. This is shown by the graphs A, B and C in Figure 28. However, by adding

less catalyst and accelerator, the actual rate of increase in temperature of the resin mass after it has gelled is slowed down. Consequently it is possible to polymerise comparatively large blocks of resin without excessive heating and the inevitable cracking which follows, provided that very small quantities of catalyst and accelerator are added, and the resin is efficiently cooled. But the cooling must be sufficiently effective to prevent the temperature of the resin block ever exceeding 25°C, as otherwise the reaction will almost certainly get out of control.

Although the heat-producing phase of polymerisation of the resin is of relatively short duration, after this is completed the hardness of the resin continues to increase gradually for at least eight days at normal room temperature. The maturing process can be accelerated, without adverse affects to the properties of the resin, by placing it in an oven at about 45°C.

The setting of the resin is inhibited both by air and water, so that any surface which is in contact with either air or water during polymerisation remains permanently tacky. The depth to which the tacky surface extends depends chiefly on two factors, the working life of the mixture and, in the case of water, the length of time the resin is in contact with water before it gels. Under average conditions, the tacky layer is quite a thin film, which can be removed by wiping the surface of the resin, when it is fully hardened, with a wad of cotton wool soaked in acetone. Acetone however should be applied to the resin only for a brief period as it has a damaging effect if applied for any length of time.

The fully matured resin is considerably harder than Perspex, but much less tough and less flexible. In colour it is a very pale yellow, but quite transparent. The exact colour depends on the conditions under which polymerisation takes place. If it polymerised slowly, with a fairly high catalyst content, but with rather a small amount of accelerator, the bleaching effect of the catalyst results in less colour than when polymerisation is comparatively rapid, with less catalyst and more accelerator. If polymerisation takes place in sunlight the bleaching effect is very striking.

Thin filaments of fully matured resin are very brittle when dry, while stout rods of it are strong and rigid. Flexibility increases noticeably if the temperature is raised to about 50°C or higher. When soaked in water the resin absorbs a small amount, and this also greatly increases its flexibility. Thin filaments of wet resin are comparatively flexible at room temperature. When placed in water at about 40°C or higher, they become very flexible. If immersed in boiling water until heated right through, even a comparatively

thick rod of resin can be easily bent to a limited extent by hand. If held in this position until cold, the rod retains its bent shape. Unlike ¼ inch diameter Perspex rod, if a similar resin rod is bent through a wide angle, it is very likely to break, as it has little tensile strength when heated sufficiently (*i.e.* to about 140°C) to be really flexible.

A more flexible resin can be produced by adding about 10 per cent Crystic resin No. 182. This plasticising resin has a clear pale brown colour, so its addition affects the colour of the 26 C resin appreciably. But models made with resin plasticised in this way are much less likely to break when dropped, than those made with unplasticised resin. The use of the plasticiser is recommended only for casting models. The addition of 10 per cent Crystic resin 182 approximately doubles the working life of the mixture.

The specific gravity of fully hardened resin is approximately 1.2. This is greater than that of resin which has just gelled, as approximately 2 per cent shrinkage takes place while the resin is maturing.

The resin is very durable and is reputed to last indefinitely without undergoing any further change once it has matured. There is no known solvent for it at present. It exhibits a high degree of resistance to the following reagents : concentrated hydrochloric acid, 50 per cent sulphuric acid, 95 per cent ethyl alcohol, benzene, formalin. When left in these reagents at room temperature for one month, the resin appears to be unaffected, provided that it was *fully matured* before the test was started.

The resin is attacked by the following reagents. Concentrated sulphuric acid corrodes it fairly rapidly. Concentrated nitric acid rapidly causes swelling and flaking; dilute nitric acid also attacks the resin, but less rapidly. A concentrated solution of sodium hydroxide rapidly causes swelling and flaking; a dilute solution attacks the resin slowly. Acetone causes swelling and crumbling of the resin.

The resin can be coloured before use by the addition either of Crystic pigment pastes, or Crystic powdered pigments, supplied by the manufacturers of the resin. The former have been ground thoroughly into a low-viscosity resin, and are used by adding about 2 per cent to the resin mixture, if an intense colour is required. If a light tone is desired, less paste is added. The paste mixes perfectly with the resin, as the particles of pigment are fine and well dispersed. These pigment pastes however are available only in a limited range of colours.

Powdered pigments are available in a wider range of colours, but in order to disperse the particles, which tend to cling together in lumps, the

powder must be ground into a paste with a little monomer, before it is added to the resin. After very thorough stirring the mixture is left for one hour in the beaker in which it was made up. It is then decanted into another beaker. Any large particles of pigment remain as a sediment at the bottom of the first beaker.

Some pigments have an accelerating effect on the polymerisation of the resin, while others have a retarding effect; but provided that not more than 1 per cent pigment powder, or 2 per cent pigment paste is added, this effect is in most cases negligible.

The resistance of the various pigments to the concentrated hydrochloric acid used to destroy the organic tissues in corrosion casting techniques varies greatly. Provided the technique concerned is not one in which it is necessary for the resin to remain in strong acid for more than three days, the protection provided by the resin prevents the acid from causing much discoloration. However, as the resin is slightly permeable to water, it is also permeated by such aqueous solutions as hydrochloric acid. Consequenly if immersion in strong acid is prolonged, discoloration is produced at ever-increasing depth from the surface if pigments were used with little resistance to the acid.

The importance of the discoloration is not always proportional to the depth to which it extends. It also depends on the nature of the discoloration. For example, red pigment paste B 214 is discoloured to a depth of 0.1 mm. after immersion of a block of resin in concentrated hydrochloric acid for a month at normal room temperature. After this period the surface of the resin is a dull grey colour. Although in the case of yellow paste B 258 the colour is destroyed to a depth of 0.2 mm. in the same period, the appearance of the block is little altered as a skin of transparent resin is left, through which the colour of the deeper layer shows.

Vermilion (mercuric sulphide) can easily be mixed into resin, if it is first wetted with monomer. Although it gives a brilliant colour, and is completely resistant to hydrochloric acid, it should not be used to colour resin, as subsequent exposure of the resin to sunlight causes comparatively rapid darkening, even if the red resin is embedded in an outer cast of transparent resin. For techniques in which particularly good resistance to concentrated hydrochloric acid is required, red lake pigment powder M 11 and blue pigment powder M 21 are recommended. These pigments also give a very pleasant contrast of brilliant colours.

The resin can be sawn, either by hand or on the band saw. It can also be filed, and ground with abrasive papers such as waterproof carborundum

scalpel, though at this stage care is required to avoid pulling the new resin away from the old. When fully hardened the new resin can be filed, ground and polished.

Before attempting to polish resin it should be smoothed with progressively finer grades of waterproof carborundum paper, finishing with grade 500 A. If a rectangular block is being smoothed the coarser grades can be used by fixing the sheet, by means of a strip of Perspex and a screw clamp, to a piece of plate glass resting on the table. The clamp clasps the edge of the table, the plate glass, the sheet of abrasive, and the strip of Perspex, so that all are held firmly, and the block is rubbed on the abrasive surface. Although the standard size of abrasive paper sold in the shops in England is 10 ins. x 12 ins. or even smaller, sheets can be ordered from the manufacturers approximately 2 ft. x 3 ft. in size. When the coarse grades are being used, large sheets are more convenient than smaller ones. But when the finer grades are used, more satisfactory results are obtained if the paper is wrapped round a block of cork. The finest grade should be applied by tearing off a small piece and pressing on it with the tip of a finger while it is being rubbed over the surface. Finally the surface is rubbed with a wad of wet cotton wool to which pumice powder has been applied, until a smooth matt surface is produced, and no scratches visible to the naked eye remain. It is important to produce a matt surface free from scratches before the resin is actually polished. Otherwise when most of the surface is polished, the scratches become conspicuous.

The resin is more tedious to polish than Perspex, as it is harder. Polishing can be done either on a linishing machine, or on a buffing wheel to which a suitable polish has been applied. An excellent finish can be produced if the final polishing is done with a swansdown mop.

In certain techniques the appearance of the cast can be greatly improved by giving it a coat of transparent resin, applied either by dipping, painting or spraying. If resin 26 C were used for this, the coat would never set but remain permanently sticky. Therefore Marco resin 28 C, which sets without a tacky surface in the presence of air, is used instead.

Marco resin 28 C is somewhat similar in appearance to resin 26 C, but much less viscous. It is used in conjunction with monomer C, catalyst H C H and accelerator E. The mixture is made up in the same way as for the other resin. This resin is not suitable for general casting work, as it sets into a much harder polymer than 26 C, and so is more liable to crack. Also it is not sufficiently viscous for some applications. Although reputed to set with a completely tack-free surface, in the case of thin films of the resin applied as

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paper. These operations should always be carried out with the resin well wetted, as otherwise the room is filled with resin dust.

A suitable resin mixture may be used as a cement for sticking two pieces of resin together. For this purpose a very viscous, rapidly-setting mixture is the most satisfactory. The following formula is recommended :

resin 26 C	100 g.
monomer C	10 g.
catalyst H C H	5 g.

The ingredients are mixed as described on page 97. The mixture will then keep in the refrigerator in serviceable condition for at least a week. During this period the viscosity gradually increases and the working life decreases.

When cement is required, a little of the mixture is placed in a small porcelain palette and one or two drops of accelerator E added and stirred in. The two ends of resin to be joined are brushed with acetone to soften their surfaces and then immediately wetted with the resin cement, so that it has the maximum time to soak into the two surfaces to be joined before it gels. The two surfaces are held in apposition (this can often be done conveniently by means of Plasticine) and more cement is applied to the gap between them when the resin is on the point of gelling, and consequently so viscous that little or none runs out of the joint. If coloured resin is being joined, the cement may be coloured also.

The time the cement takes to gel depends on the amount of accelerator added and the age of the cement. This time should not be longer than fifteen minutes, unless the room temperature is well below 20°C, or too little accelerator has been added. The working life of the cement can be substantially reduced by warming it. This can conveniently be done by directing on to it hot air from a hair drier. But care and experience are needed to avoid warming the cement too much, as the immediate effect is to make it less viscous and therefore more liable to run out of the joint, while the subsequent effect is to cause it to gel so rapidly that it may solidify before the joint can be properly filled with it.

It is equally simple to add to a piece of resin, by the use of the cement. A cavity is built by surrounding the place where it is desired to add resin, with Plasticine, which must be pressed very firmly against the resin to prevent the escape of cement between the resin and the Plasticine. When the resin cement is about as hard as Cheddar cheese, it can be trimmed with a sharp

scalpel, though at this stage care is required to avoid pulling the new resin away from the old. When fully hardened the new resin can be filed, ground and polished.

Before attempting to polish resin it should be smoothed with progressively finer grades of waterproof carborundum paper, finishing with grade 500 A. If a rectangular block is being smoothed the coarser grades can be used by fixing the sheet, by means of a strip of Perspex and a screw clamp, to a piece of plate glass resting on the table. The clamp clasps the edge of the table, the plate glass, the sheet of abrasive, and the strip of Perspex, so that all are held firmly, and the block is rubbed on the abrasive surface. Although the standard size of abrasive paper sold in the shops in England is 10 ins. x 12 ins. or even smaller, sheets can be ordered from the manufacturers approximately 2 ft. x 3 ft. in size. When the coarse grades are being used, large sheets are more convenient than smaller ones. But when the finer grades are used, more satisfactory results are obtained if the paper is wrapped round a block of cork. The finest grade should be applied by tearing off a small piece and pressing on it with the tip of a finger while it is being rubbed over the surface. Finally the surface is rubbed with a wad of wet cotton wool to which pumice powder has been applied, until a smooth matt surface is produced, and no scratches visible to the naked eye remain. It is important to produce a matt surface free from scratches before the resin is actually polished. Otherwise when most of the surface is polished, the scratches become conspicuous.

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a finishing coat, the surface may remain very slightly tacky for several weeks or even months. Provided that the cast is protected from dust after being sprayed, this slight tackiness does not matter. But if it is desired to avoid any trace of tackiness, this can be achieved by placing the cast in an atmosphere of carbon dioxide before the resin sets and leaving it in this for several hours until the resin coat is quite hard. Full practical details concerning the use of this resin as a finishing spray are given in the next chapter.

The resins, pigment pastes (which are made up in resin), monomer C and accelerator E have a limited shelf life. Provided that the temperature never exceeds 20°C (68°F) the resins will keep for about a year, if stored in metal tins (*i.e.* light proof). Bright daylight reduces the shelf life considerably, while direct sunlight may cause the resin to gel in a few days.

Monomer C and accelerator E have a shelf life under similar conditions of between three and six months; but if the temperature of any of these materials is allowed to rise above 20°C, the shelf life is substantially reduced.

Catalyst H C H and the powdered pigments keep indefinitely.

If stored in a refrigerator at a temperature of about 5°C or less, the serviceable life of these materials is very greatly extended. Under these conditions all keep in good condition for at least a year.

The resin remains usable as long as there are no gelatinous lumps in it, and as long as there is no noticeable increase in viscosity. The monomer must only be used while it is a really mobile fluid. If it becomes even slightly viscous, it should be discarded, as it no longer mixes completely with the resin. The accelerator must be used only if it is a mobile violet fluid. If its viscosity increases to that of a thin oil, or if it turns green, it must be discarded. Although in the latter condition it promotes polymerisation, it makes uncoloured resin cloudy. A further test for the accelerator is provided by running a little of it from a pipette into a beaker of acetone. If it is in good condition, the accelerator mixes instantly with the acetone, yielding a perfectly clear liquid; otherwise it produces a cloudy liquid.

The resin, monomer and accelerator must never be exposed to sunlight, even for a few minutes. Sunlight not only reduces the shelf life of the components, but greatly accelerates polymerisation of resin mixtures.

Each resin mixture is made up in a beaker, and the components must be very thoroughly mixed. This can be achieved more efficiently by stirring with a strip of Perspex, than with a glass stirring rod. Not only is the mixing more efficient, but, no matter how vigorously the mixture is stirred, there is

no risk of breaking the beaker. The edges of the Perspex strip should be polished, to facilitate cleaning after use.

The resin mixtures are extremely messy substances to handle. They are best removed from the hands and apparatus either by acetone, or by a suitable liquid toilet soap, such as Homacol (see Appendix). In each case the reagent is applied on a wad of cotton wool, which should be used only once. Acetone is applied directly to the cotton wool, but when liquid soap is used the cotton wool is first soaked in water and wrung out. Acetone must not be used to clean Perspex, as it attacks the latter. Pipettes used to measure the accelerator are cleaned in acetone, as the accelerator is freely soluble in this reagent. Glassware contaminated with resin which contains catalyst and accelerator is best cleaned by first allowing the resin to gel, and then boiling in water containing about 1 per cent Homacol for an hour. After the water has cooled to the room temperature, the hardened resin comes away easily from the glass. Resin which sets in rubber tubing can be expelled about half an hour after the resin has solidified, before it gets really hard, by connecting one end of the tubing to the tap, and running water through it. The water stretches the rubber so that the resin is freed and then expelled. The inside of the rubber tube is cleaned by forcing through it, by means of water from the tap, a number of small wads of cotton wool soaked in acetone.

Owing to the limited shelf life of the components of the resin mixtures when stored at a temperature which never exceeds 20°C, and the very much reduced life of the monomer and accelerator if exposed to higher temperatures, even for a few hours, these materials cannot be transported and marketed by ordinary retailers. They must be ordered direct from Scott Bader & Co. (see Appendix). This firm will supply the materials directly, or arrange for their supply from a company manufacturing them under licence.

The materials are not normally supplied by the manufacturers in the comparatively small quantities needed for this work. They have made up a special anatomical kit for those who wish to do this work, and have included in this kit suitable quantities of the various components which may be required, for use with a unit of 4 quarts (12 lb.) of Marco resin 26 C.

If additional components are required, the worker should contact the firm of Scott Bader & Co. Ltd. before ordering, to find out in what units these should be ordered, to facilitate packing and dispatch.

The transport of these materials to some parts of the world is costly, as it is essential that they travel to hot countries in cold storage, or by air by night service. The risk of the materials being ruined in transport, or of their

subsequent shelf life being greatly reduced, can in some cases be eliminated by ordering them during the winter. Provided that they arrive in perfect condition, and are kept in a refrigerator until required, they will remain in perfect condition for at least a year. The resin itself will keep in the refrigerator for over two years.

Chapter 17

THE GENERAL PRINCIPLES GOVERNING THE USE OF THE RESIN FOR CASTING

1. INTRODUCTION

THE purpose of this chapter is to describe the practical details of the general procedure common to many specialised techniques, so that it may be omitted in subsequent chapters. It is therefore essential that the worker who intends to apply any of the techniques described in subsequent chapters shall also be well acquainted with the relevant parts of the present one.

Remarkably consistent results can be obtained with Marco resin 26 C, but frequent failures will be encountered unless the reasons for the various operations are clearly understood. Although basically simple, to produce the best results this work has to be done intelligently; it is not enough just to follow blindly the instructions given. Therefore a special effort has been made to explain the reason for each detail of procedure, so that, if something goes wrong, the worker will be able to diagnose correctly the cause of failure. Then it is not difficult to avoid making the same mistake again. Those who undertake this type of work must realise that the results are not a matter of hit or miss.

2. CASTS OF BLOOD VESSELS AND DUCTS

These can be made from a limb, or such viscera as lungs, liver, kidney, spleen, brain, heart, etc. A short length of Portex polythene tubing (or Portex vinyl V.Y. standard tubing for sizes larger than 7.5 mm. internal diameter) is first tied very securely into the end of each of the vessels or ducts which are later to be filled with resin. Linen carpet thread is ideal for tying the cannulae in position, as it is very strong but does not cut into the tissues. The largest size of cannula which fits easily is used, as this facilitates the flow of the relatively viscous resin used for all the injections. Care is sometimes needed, when inserting cannulae into the vessels of organs taken from old subjects, to avoid pushing in a piece of the intima (the inner wall of the vessel), so that it forms a plug inside the vessel, as in many old subjects the attachment of the intima to the outer wall of blood vessels is extremely weak. Although such

a plug may not obstruct the vessel sufficiently to impede the injection of fixative, it may seriously retard the flow of the resin.

Portex polythene tubing is preferable to glass for several reasons. Although the former is semi-rigid, its limited flexibilty facilitates manipulations during the injection, but its principal advantage is that, unlike glass cannulae, if a polythene cannula is securely tied in, it rarely slips out. This point is particularly important as, when a resin injection is being undertaken, in order to obtain the best results it is essential to inject the resin at the latest possible moment before it is expected to gel. Consequently if a cannula slips out during the injection there is seldom time to reinsert it before the resin gels. Polythene cannulae can be cut through with ordinary scissors, soon after the resin has solidified. When the resin is really hard a polythene cannula can be easily removed, as the resin does not stick to it. A glass cannula may have to be broken before it can be removed from the cast.

Portex polythene tubing cannot be directly attached to other apparatus, owing to its lack of elasticity. Consequently the cannula is first attached to a short length of rubber tubing, of internal diameter such that the latter can be easily connected to whatever apparatus is used subsequently for washing out the vessels, fixing the material, and for the injection of the resin. If the polythene cannula is too slender to fit firmly into the rubber tubing, the external diameter of the former is increased by rolling a suitable length of zinc oxide sticking plaster round its end. The roll of sticking plaster is bound with button thread or Chinese twist before it is inserted into the rubber tube, as otherwise the roll may come undone. The outside of the joint is firmly tied with fine string. Figure 2, p. 7 illustrates the method of connecting a polythene cannula to a length of rubber tube . The polythene cannula, with the rubber tube attached to it, is not removed from the vessel until the resin injection has been completed. If in subsequent work apparatus is used which requires either a larger or a smaller size of rubber tube to connect it, the simplest way of making the joint is to select a size of rubber tube which will either fit outside or inside the rubber tube already attached to the cannula. After the joint has been made by sliding one piece of tubing inside the other, it is firmly tied with fine string. If properly made, such joints are quite reliable, even if a considerable injection pressure is later exerted.

After cannulae have been tied into all vessels and ducts which are later to be filled with resin, the organ is immersed in cold water and manipulated so as to remove any air trapped in the vessels and cannulae. Adjustable screw clamps (see Fig. 2, p. 7) are then fitted to the rubber tubing attached to each

of the cannulae, and these are subsequently always tightened before the organ is removed from fluid, to prevent any air from entering, and causing air locks which may obstruct the flow of the resin.

Next, the blood is washed out of the vessels later to be filled with resin. In the case of organs, this is done by injecting a large quantity of cold deaerated water into the arteries and allowing it to escape via the veins. It is also desirable to wash out ducts such as the bile duct with water. In the case of a limb normal saline must be used instead of water, as the latter makes the part waterlogged. Sodium citrate must not be used, as the citrate inhibits the setting of the resin. It is necessary to deaerate the water before injecting it, as tap water is frequently supersaturated with air, some of which otherwise comes out of solution in the vessels which are being washed out. The water can be conveniently deaerated in a 15 litre aspirator, by means of the water vacuum pump. It can be run into the vessels directly from the aspirator by gravity flow in those cases where quite a low pressure is needed, or by means of an enema syringe, if a greater injection pressure is necessary.

Although it is not essential to fix the organ before injecting it with resin, it is advisable to do so (except in the case of the spleen) for several reasons. The organ is easier to handle after fixation and it keeps its shape better as a result of slight hardening of the tissues. But the most important advantage is that the injection of the resin can then be undertaken at leisure, and as much preparation as possible made the day before, so that the actual injection is unhurried. The material may be fixed either with 5 per cent formalin, of 70 per cent spirit; but if spirit is used, all traces of it must be washed out before the resin injection is made. Failure to do this may result in resin soaking excessively into the walls of vessels. If this happens, the cast of the cavities of the vessels is marred by resin-impregnated walls of vessels adhering to it in places. *A limb must not be fixed in spirit, if the vessels are to be filled with* resin, as it is impossible to wash out all the spirit before the resin injection is undertaken.

It is not advisable to leave the fixed material longer than a week before injecting the resin, as in some cases this leads to excessive hardening of the tissues, so that the flow of the resin is impeded. While the material remains in the tank of fixative, some of the latter is injected by means of an enema syringe every other day into all vessels or ducts later to be filled with resin. This prevents small particles of clotted blood collecting into comparatively large clots, which would obstruct the flow of the resin.

Spirit is washed out of an organ, the day before the resin injection is

In most cases the resin mixture used for filling vessels and ducts should have a working life of about fifteen minutes, as this gives sufficient time to complete the injection, and yet the mixture does not heat up excessively before it gels (see Fig. 28, p. 100). The only exception to this rule is when a cast is required of exceptionally fine vessels. In this case the most important consideration is to reduce the inhibition due to water to a minimum. This is achieved by using a mixture with a working life of ten to eleven minutes.

Before resin mixtures are made up, the formula of each, based on units of 100 g. resin, and multiplied by the necessary factor to give the total quantities required, should be written down and carefully checked.

Before the actual injection, a careful test should always be made to determine as accurately as possible the working life of the particular resin mixtures to be used. If more than one system is to be injected, the injection should be so timed that all the different resins injected gel at the same moment. For example, if the tests indicate that one mixture has a working life three minutes longer than the other, the accelerator is added to the mixture with the longer working life three minutes earlier than to the one with the shorter working life. Then the two mixtures will gel simultaneously.

The test to establish the working life of a resin mixture is made in the following way. Suppose the following mixture is to be used :

Marco resin 26 C	100 g.
Monomer C	15 g.
Catalyst H C H	4 g.
Pigment paste	2 g.
Total weight	121 g.
Accelerator E	4 ml.

If it is estimated that, to allow for resin in the apparatus when the injection is completed, wastage by leakage etc., 300 ml. resin mixture are required, 4 units of the above mixture are prepared. Then one unit, *i.e.* 121 g., is weighed out into a beaker and, shortly before the injection is made, one unit of accelerator, *i.e.* 4 ml., is added to this test sample and very thoroughly stirred in. The working life of this sample is determined, care being taken to ensure that it polymerises under the same conditions (particularly with regard to temperature) as those under which the rest of the mixture will be used. When the accelerator is being stirred in, the beaker containing the resin should

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never be clasped in the palm of the hand, as this may cause it to be warmed up several degrees above room temperature.

In the case of the resin mixture used for the injection, after the accelerator has been added and thoroughly stirred in for two minutes, the mixture is allowed to stand for a minimum period of two minutes in the beaker in which it was made up, to allow the larger air bubbles to rise to the surface, before it is transferred to the injection apparatus. Small air bubbles remaining can be ignored in this type of work.

Provided that the injection apparatus is connected to the vessels, and its position carefully adjusted before the accelerator is added to the various resin mixtures to be used, mixtures with a working life of fifteen minutes allow adequate time to complete all vascular injections before the resin gels, though in some cases, as for example a quadruple injection of the liver, or a triple injection of the lungs, it is necessary to have two operators working as an efficient team, to mix in the accelerator, and fill the apparatus with resin. Before attempting a triple or quadruple injection for the first time, it is advisable for the two workers to rehearse the whole procedure in the greatest detail. And before the final work, which starts when the accelerator is added to the resin mixtures and terminates with the gelling of the resin, is commenced, the exact times at which the accelerator is to be added to each resin mixture, the time at which the injection is to be commenced, and the time at which the resin is expected to gel, should all be clearly written down, and placed in a prominent place on the work bench, so that they can easily be referred to. It is unwise to trust these vital facts to memory.

As the resin is left in the injection apparatus until it solidifies, the apparatus must either be so constructed that solid resin can be removed from it or so cheap that it can be thrown away after use, as there are at present no solvents available to dissolve the resin once it has solidified.

In certain specialised techniques a relatively large amount of resin is injected at a very low injection pressure. In these cases the most suitable apparatus consists of a glass or polythene funnel, which is held by means of a retort stand and clamp between six and twelve inches above the organ which is to be injected, and connected by means of rubber tubing, as shown on the left side of Figure 29. But in the majority of cases where blood vessels or ducts are to be filled with resin, only a relatively small quantity of resin is required to fill them, and an apparatus must be used which allows the volume of resin injected to be observed more accurately than is possible when a funnel is used, as this gives the most reliable indication of the success of the

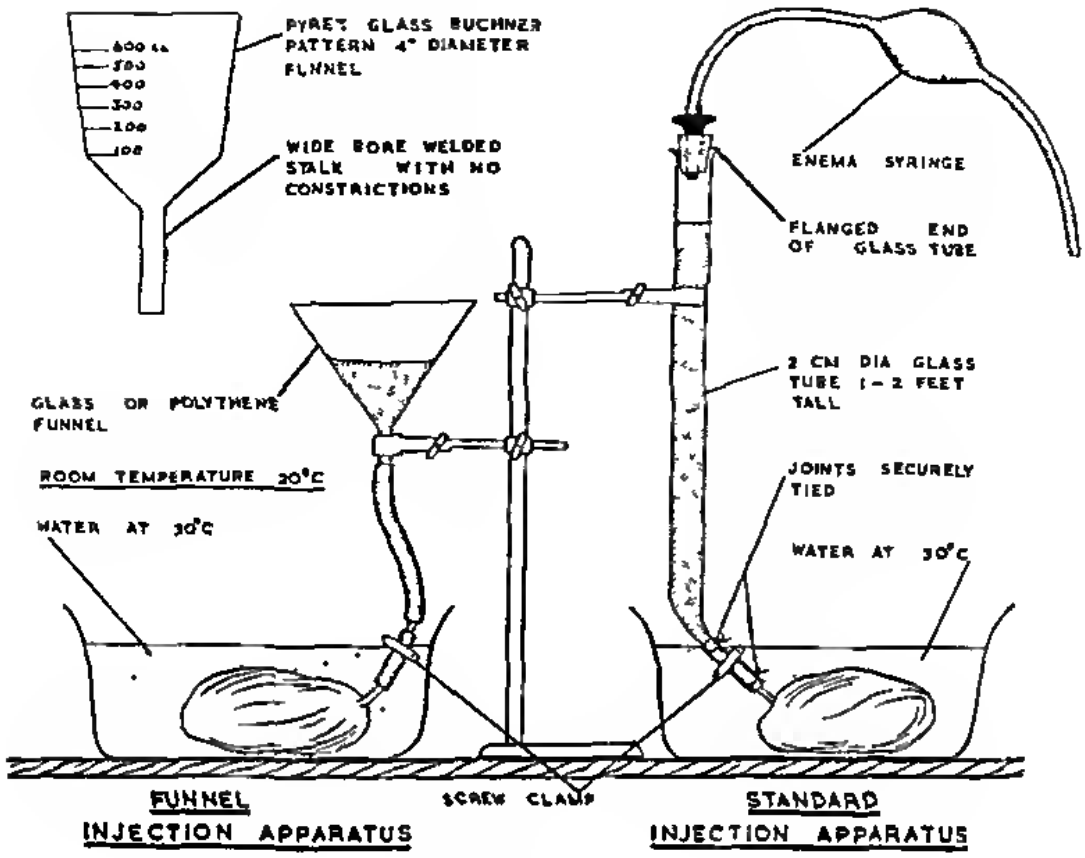

FIG 29

Two types of apparatus for the injection of resin into anatomical specimens The funnel type is used where a relatively large volume of resin is to be run in at a very low injection pressure A special type of funnel is also illustrated, which is particularly suitable for making casts from the lungs This funnel consists of a sintered glass filter, before the filter has been fixed into it These funnels can be ordered from the makers of sintered glass funnels

The Standard injection apparatus is used where relatively small quantities of resin are to be injected It enables a considerable injection pressure to be applied by means of an enema syringe The flow of quite a small quantity of resin is indicated clearly by the fall in the level of the resin in the glass tube

injection. There must also be some method by which, when the progress of the injection is inadequate by gravity flow alone, the flow of resin can be accelerated by the immediate application of considerable additional injection pressure.

A simple apparatus which fulfils these requirements is shown on the right side of Figure 29, and is referred to for convenience as the Standard injection apparatus. In this apparatus the resin reservoir consists of a one to two feet length of glass tube, of 2 cm. internal diameter, which is held vertically by means of a retort stand and clamp. The lower end of the tube is drawn out so that it fits into whatever rubber tubing is already attached to

the polythene cannula. This end is slightly flanged so that a really secure joint can be made when the rubber tube is tied to it. In some cases the adjustment of the glass tube is facilitated if its lower end is bent.

When this apparatus is used, gravity flow is often adequate to obtain a complete injection, though to maintain sufficient injection pressure by this means a fairly long tube may have to be used, and it may be necessary to top up the resin in it frequently to maintain an adequate head of pressure. But additional pressure can be exerted by pumping air into the top of the glass tube by means of an enema syringe. The delivery nozzle of the enema syringe is inserted into a hole passing through the centre of a rubber bung which fits the tube. When pressure is required to accelerate the flow of resin, the rubber bung is pressed into the top of the glass tube and held in position with one hand, while the bulb of the syringe is operated with the other. The rubber bung should not be tied in position, owing to the delay caused in removing it, if it is necessary to top up the resin in the glass tube. A single enema syringe can be used to accelerate the flow of resin in several tubes which are being used at the same time, by alternately applying pressure first to one and then to the other. A clear warning is given of excessive pressure by the greatly increased effort needed to compress the bulb of the syringe, and by back-flow of resin into the glass tube, when the pressure is released by removing the rubber bung by which the syringe is connected to the glass tube.

As it would be impossible to apply injection pressure by means of the enema syringe if the top of the glass tube were split when the rubber bung was pressed into position, the rim of the tube is slightly opened after the glass has been softened in the flame. This ensures that the bung does not press against the rim of the glass tube, and reduces the risk of the glass splitting.

The injection is made in the following way. First the clamp attached to the rubber tube which connects the polythene cannula to the injection apparatus is closed. Surplus water in the end of the rubber tube is tipped out, and the tube is attached to the injection apparatus. If there is any possibility of injection pressure being later exerted by means of the enema syringe, all joints in the rubber tubing must be securely tied. The apparatus is carefully adjusted so that the organ just touches the bottom of the sink or basin in which it is immersed in warm water. The temperature of the water is checked and adjusted if necessary. An inspection is made to see that no vessels are either twisted, or dragged by the injection apparatus out of their natural position. The temperature of each of the resin mixtures is checked and, if found to

be above room temperature, it must be cooled down to this level before use, or it will have a shorter working life than the test sample.

When everything is ready, and the time at which the accelerator is to be added to each resin mixture, the time at which the injection is to be commenced, and the time at which all resin mixtures are expected to gel, have been written down in such a way that these figures can be instantly seen, previously measured quantities of accelerator E are added to each of the resin mixtures and very thoroughly stirred in for two minutes. Then the mixtures are allowed to stand for as long as possible before being poured into the injection apparatus. This not only gives the maximum time for air bubbles in the resin to rise to the surface, but avoids the risk of premature gelling of resin in the rubber tubing close to the specimen, which might occur if the resin were poured into the injection apparatus several minutes before the injection was due to be commenced, owing to this tube being immersed in warm water.

After the injection apparatus has been filled with resin, the rubber tubing is compressed in such a way as to expel any air trapped in it. The injection is commenced by releasing the clamps. If several different-coloured resin mixtures are being injected, as for example in a quadruple injection of the liver, it is advisable to start all simultaneously. If it appears necessary, additional pressure is applied by means of the enema syringe. The tubes are topped up if the level of the resin falls considerably. A check is made to see if there are any serious leaks of resin, which can be controlled by the application of artery forceps. The progress of the injection is judged by the quantity of resin which flows into the organ, due allowance being made for that required to fill the injection cannula, and any resin which has been lost by leakage. A fall of 1 cm. in the level of the resin in the glass tube represents an injection of approximately 3 ml. When filling large vessels, a low injection pressure is always adequate, and the unnecessary application of additional pressure makes the organ so impregnated with resin that it is difficult to remove the macerated tissue from the cast of the cavities of the vessels. But in the case of small vessels a much higher injection pressure is necessary, and there is no risk of undesirable consequences resulting from the use of an unnecessarily high pressure.

There are two methods of establishing when the initial injection has been successfully completed: by previous knowledge of the approximate quantity of resin required to fill the vessels, and by a sharp falling off in the rate of flow of resin, even when a considerable pressure is exerted (in the case of

small vessels). The apparatus is left connected until the resin has gelled so that, after the initial injection has been completed, resin lost by leakage and through soaking into the tissues may be replaced by gravity flow from the reservoir of resin.

About half an hour after the resin has gelled, the polythene cannulae are cut through with a strong pair of scissors. If the Standard injection apparatus has been used, the glass tubes, now filled with solid resin, are discarded. It is possible to expel the resin from the rubber tubing, if the labour of salvaging this for future use is considered worth while (see p. 107). If polythene funnels were used as reservoirs for the resin, they must be immersed in cold water immediately the resin in them feels hot to the touch, as otherwise the heat generated by the resin during this phase of the polymerisation will ruin them. After the resin is quite hard, it can be easily removed from polythene funnels. Resin can be removed from glass funnels after it has set, provided the latter do not have any constriction in their stalks.

In the case of an injected limb, which is to be dissected, this work can be commenced two days after the resin injection has been completed. When such a specimen is dissected, the walls of the vessels can be stripped completely away, leaving a coloured cast which is much more conspicuous than vessels filled with a coloured mass.

If the tissues are to be corroded away, the specimen must be left for at least eight days before it is placed in acid, as otherwise the acid used to dissolve the tissues attacks the resin also. During this period the specimen is left in water in a fairly warm room. Maceration in acid is quicker if some decomposition takes place during this period. But if it is necessary for the specimen to remain in a room where the unpleasant smell would be objectionable, decomposition can be prevented without risk of damage to the resin by keeping the injected specimen in 2 per cent formalin.

The tissues of the organ are dissolved by immersion in concentrated hydrochloric acid. Either a glass accumulater jar or a Perspex box provides a suitable container for the acid. The organ is lowered into the acid on a Perspex tray, with Perspex handles projecting above the acid level, as this facilitates lifting the macerated specimen out of the acid without the risk of damage to some delicate part. When first immersed, all organs float in the acid. They should either be turned over from time to time until they sink, or else totally immersed by placing suitably bent strips of Perspex over them to weight them down, as otherwise maceration of the part projecting above the acid is considerably delayed. A well-fitting cover must be provided for

the acid bath, as acid fumes cause severe rusting to dissecting instruments, even if they are made of stainless steel. This happens even when the concentration of acid fumes is far less than that necessary to cause any discomfort to those working in the laboratory.

Although concentrated hydrochlorie acid has no effect on fully matured resin, it slowly attacks some of the pigments used to colour the resin. Therefore an organ injected with coloured resin should only be left in the acid bath for the minimum period necessary for the organic tissues to be fully macerated. This varies from twenty-four hours to several days, according both to the size of the organ, and to whether the acid is full strength, or has been weakened by previous use.

When the organ appears to be completely macerated, at least on the outside, it is removed from the acid bath, and immersed in cold water in the sink, with the cold tap left on. A length of rubber tube is attached to the delivery nozzle of both the cold and hot taps, to avoid splashing and to enable jets of both hot and cold water to be directed as required during the washing of the east. Before this work is undertaken the hands should be protected by a suitable barrier cream such as Innoxa B.W.2. This is the same cream as is recommended for protection against formalin. If applied carefully in strict accordance with the manufacturers' instructions, a high degree of protection is afforded against hydrochloric acid.

The macerated tissues are washed from the cast by directing a jet of hot water (or, in the case of delicate casts, cold water) on to the specimen while it is held immersed in cold water. The force and size of the jet are regulated by adjustment of the tap, and by partially compressing the end of the rubber tube attached to the latter. The most powerful jet is used which does not cause serious damage to the cast. The best results are usually obtained by directing the jet from above the level of the water in which the specimen is immersed. This causes the jet of water to draw down a large amount of air, which helps to float away the macerated tissues detached from the cast.

Washing is continued until no more macerated tissue can be removed from the specimen. If the water in the sink becomes too dirty to observe the cast clearly, and to see immediately if the jet of water is causing damage, the specimen is removed and placed in a basin of cold water, while the sink is emptied and refilled with clean water. During the final stage of washing, it is sometimes advantageous to fit a glass cannula to the end of the rubber

tubing, so that a strong jet of water can be directed on one particular area at the centre of the specimen.

Incomplete maceration is indicated by the presence of recognisable tissues, which cling tenaciously to the cast, even after thorough washing. An incompletely macerated specimen is returned to the acid bath for a further twenty-four hours, and then washed again.

If the original tissues have been impregnated with resin, they cannot be removed by washing alone, even after complete maceration. Resin-impregnated tissue is converted by the acid into a substance of crumbly consistency, which has to be dissected away. While this work is being done, the cast is held over a basin of warm water by means of a clamp and retort stand, and is kept wet by frequent basting with water. If it is desired to increase the flexibility of the resin, or soften the material being dissected away, the temperature of the water is increased to 40°C. It is unwise to use water hotter than this, as it may result in the cast becoming warped. However, if this should happen, the cast can be restored to its original shape, by immersing it in very hot water until it is quite flexible, and then by holding it in the desired position while it cools and becomes rigid again.

The dissection of macerated tissue is carried out by digging it away with a very large needle fixed to a handle (see p. 42). McIndoe's dissecting forceps (Fig. 9, p. 29) are also very useful for this work. After some of the crumbly tissue has been removed, a great deal more will have been loosened. This is removed by washing the cast.

Whether or not any dissection is necessary, casts made of synthetic resin always require extensive pruning, as there is no way of making the injection self-limiting. Resin always fills some of the finer vessels and the casts of these have to be pruned away to show the larger ones. The pruning can usually be done with a pair of long curved opthalmic scissors (see Fig. 8, p. 28), with the cast kept wet with water at about 35°C. If cold water is used, not only may the resin be inconveniently hard to cut with scissors, but the little pieces cut off are liable to fly into the pruner's eye. In some cases it is easier to prune the cast by breaking off the twigs with dissecting forceps. It is hazardous to do such pruning by pulling the twigs away; they should be broken by bending them at right angles to the main branch. Until the pruning has been completed, the cast should be left at night immersed in water, to protect it from damage.

When both dissection and pruning are necessary, the normal practice is first to dissect the outer tissues, and then prune this area, before proceeding

to deeper dissection, as pruning of the periphery of the cast facilitates deeper dissection.

The difficulty and labour of dissecting away macerated tissue and pruning the cast vary enormously, according to the particular organ injected, the number of systems injected in the same organ, and the skill and judgment with which the resin injection was made. For example, a triple cast of the portal vein, hepatic artery and bile duct, should require no dissection at all, if the injection was made correctly. But if a quadruple cast is being prepared, composed of the above-mentioned systems plus the hepatic veins, a great deal of dissection is unavoidable. To those inexperienced in this work the difficulty of dissecting the macerated tissues away from the cast without serious damage to the latter may in this particular case appear to be insuperable. However, all that is really needed is faith and patience. The worker must become reconciled to the idea that the dissection and pruning of the cast may take up to two weeks to complete. And such work must never be continued when symptoms of fatigue or impatience appear. These are at once apparent to an experienced spectator, by the increasing violence with which the work is being tackled; unfortunately the worker himself is not always immediately aware when the time has come to take a rest.

If any important branch of the cast is broken, it can be cemented in its original position with resin cement made up and applied as described on page 104. The two ends to be joined are dried, and the branch is held in position by means of a few small wads of cotton wool, which are supported by neighbouring branches. Considerable patience and legerdemain are needed to fix the branch in the correct position. After a branch has been repaired in this way, the cast is left overnight with the actual joint not immersed in water, as water has an adverse effect on the hardening of the cement.

When the pruning has been almost completed, there are always a large number of minute twigs which would be exceedingly tedious to remove by the method described above. The cast is therefore allowed to dry, and then the twigs, which become rigid and brittle, are broken off by pressing against them with the end of a slender probe.

The pruned cast usually requires a certain amount of cleaning. This is necessary partly because its whole surface may be covered by a film of resin, the setting of which was inhibited, and which has in consequence been attacked and discoloured by the acid. In addition, the remains of the walls of vessels, unpregnated with resin, may still adhere to parts of the cast. The

need for cleaning the cast is judged by its appearance when thoroughly wet. When dry the cast is invariably covered by a whitish bloom, which is made invisible in the last stage of the technique by spraying with a resin mixture which acts as a clearing agent.

If the appearance of the cast, even when it is wet, is not entirely satisfactory, there are various ways by which it can be improved. Particularly noticeable whitish areas are removed by scraping away the whitish surface film, but the labour required to treat the whole cast in this way would be excessive. Consequently other less effective, but also less laborious methods must be used. Each of those recommended below should be tried in turn.

Much of the surface film sometimes flakes off, if the cast is alternately immersed in water as hot as can be used without risk of damage, and then allowed to dry. The exact temperature of the water depends on the nature of the cast. If it is to have a noticeable effect this treatment may have to be repeated many times.

When no further improvement is produced by treatment with water, the same procedure can be adopted, using hot industrial spirit (95 per cent ethyl alcohol) and then cold benzene as the washing agent.

Occasionally a remarkable improvement can be achieved by immersing the cast in clean concentrated hydrochloric acid overnight; but this treatment should not be used if one of the pigments by which the resin was coloured is known to have a poor resistance to acid.

The final means by which the general appearance of the cast can sometimes be improved is by immersion overnight in hydrogen peroxide.

After the cast has been cleaned as thoroughly as possible, a suitable rod is fixed to it, by which the cast can later be mounted. As the finer branches of the casts are fragile when dry, the method of mounting must be extremely secure, to eliminate the risk of damage to the cast if the mounting came adrift. A rod made of steel piano wire, between 2 and 3 mm. in diameter, is suitable for all but the lightest casts, which can be supported by somewhat thinner brass wire. Whichever type of wire is used, this is later slid into a brass tube fixed vertically to a wooden base, and soldered in position. As piano wire cannot be soldered reliably, a thread is first cut in the upper end, and two brass screws are screwed down as far as they will go. Lated these screws are soldered to the brass tube to prevent the rod from moving.

A hole is drilled into the most convenient part of the cast to take the threaded end of the mounting rod. The cast is soaked in water for a few moments before the hole is drilled, as the cast is far less liable to damage

when being handled in this condition than when dry. One side of the threaded end of the piano wire is filed flat, so that the specimen cannot come unscrewed from the rod when cemented to it. The threaded end is cemented into the cast, using resin cement as described on page 104. If necessary the threaded end of the piano wire can be bent with pliers, if first heated in the bunsen. When brass wire is used for mounting, the end to be cemented into the cast is first hammered until it is slightly flattened, as this makes it impossible for the cast to rotate on the wire, if the cement should ever come loose.

Next the cast is sprayed with the following resin mixture :

Marco resin 28 C	100 g.
Monomer C	40 g
Catalyst H C H	2 g.
Acetone	20 ml.
Accelerator E	2 ml.

Acetone is added to the mixture for two reasons. It softens the surface of the cast and so facilitates the wetting of it by the resin mixture which is being sprayed on; and it also reduces the viscosity of the mixture, so that spraying is facilitated. The acetone soon evaporates away after the spray has been applied to the cast.

This mixture gradually increases in viscosity from the moment the accelerator is added. However it can be used for at least twenty minutes, provided a suitable spray is available, and an adequate operating force can be applied. The only spray known to the writer which is satisfactory for this work is the de Vilbiss No. 15. This spray can be obtained from surgical instrument makers in any part of the world. It is operated by removing the rubber bulb, which does not provide adequate pressure for spraying anything as viscous as the resin mixture to be used, and connecting the spray to a cylinder of carbon dioxide (or other suitable gas), by means of a length of rubber tubing. The tubing is cut in two, and rejoined by means of a glass T piece. The flow of carbon dioxide is suitably adjusted, and left on. When the spray is required, a finger is placed over the open end of the T piece, through which all the carbon dioxide otherwise escapes. The carbon dioxide is now compelled to pass through the spray and thus to operate it. As the viscosity of the resin mixture increases, it may be necessary to increase the rate of flow of the carbon dioxide.

The effect of the spray in restoring the natural colours of the resin is

quite spectacular; but great care is necessary to direct the spray in every direction in order to avoid missing some areas. The cast should be sprayed until the resin drips from it. Then for the next half hour or so, droplets which appear on the extremities of branches of the cast, but which are composed of resin too viscous to drip off, are carefully removed with a small sable paint brush. When no more droplets appear, the cast is placed in a dust free place while the resin polymerises. The spray must be emptied and thoroughly washed out with acetone before the resin sets. It is advisable to leave the metal part of the spray soaking in acetone for a few days after use, as quite a tiny drop of resin setting inside it may make it unserviceable. Owing to the limited period during which the resin mixture remains sufficiently fluid to be used as a spray, it is advisable to have a reserve spray at hand, in order to avoid delay, in case the delivery nozzle of the spray becomes obstructed.

Although Marco resin 28 C sets in contact with air with a comparatively tack-free surface, in the case of a thin film the surface remains slightly tacky for several months. Provided the cast is well protected from dust during this period, this does not matter. All traces of tackiness can be avoided if the cast is placed in a draught of carbon dioxide while the resin cement is polymerising. A large rectangular Perspex container is suitable for this purpose. A hole is drilled through one wall near the bottom of the container, into which a rubber bung is fitted. A glass tube passes through a hole in the bung. This is connected to a cylinder of carbon dioxide, the valve of which is adjusted so that a slow flow of the gas enters the container, the top of which is covered by a sheet of glass. The sprayed cast is put into the container and the lid placed in position Carbon dioxide soon replaces the air, both air and carbon dioxide escaping between the lid and the rim of the container. Although the flow of carbon dioxide may be stopped after four hours, it is advisable to leave the cast in the container overnight. A smear of heavy grease is applied to the outside of the junction between the lid and sides of the container to prevent any air entering during this period. If the resin polymerises under these conditions a completely tack-free glaze is produced.

The cast is mounted by sliding the mounting rod into a length of brass tube which is fixed vertically. The tube is fixed to a wooden base by means of a rectangular piece of brass plate, through a hole in the centre of which one end of the tube is passed and soldered. The brass plate is screwed to the wooden base. If a brass mounting rod has been used, this is soldered to the top of the brass tube. In the case of piano wire, the brass screws previously fixed to the latter are soldered to the top of the tube. Figure 30 shows the details

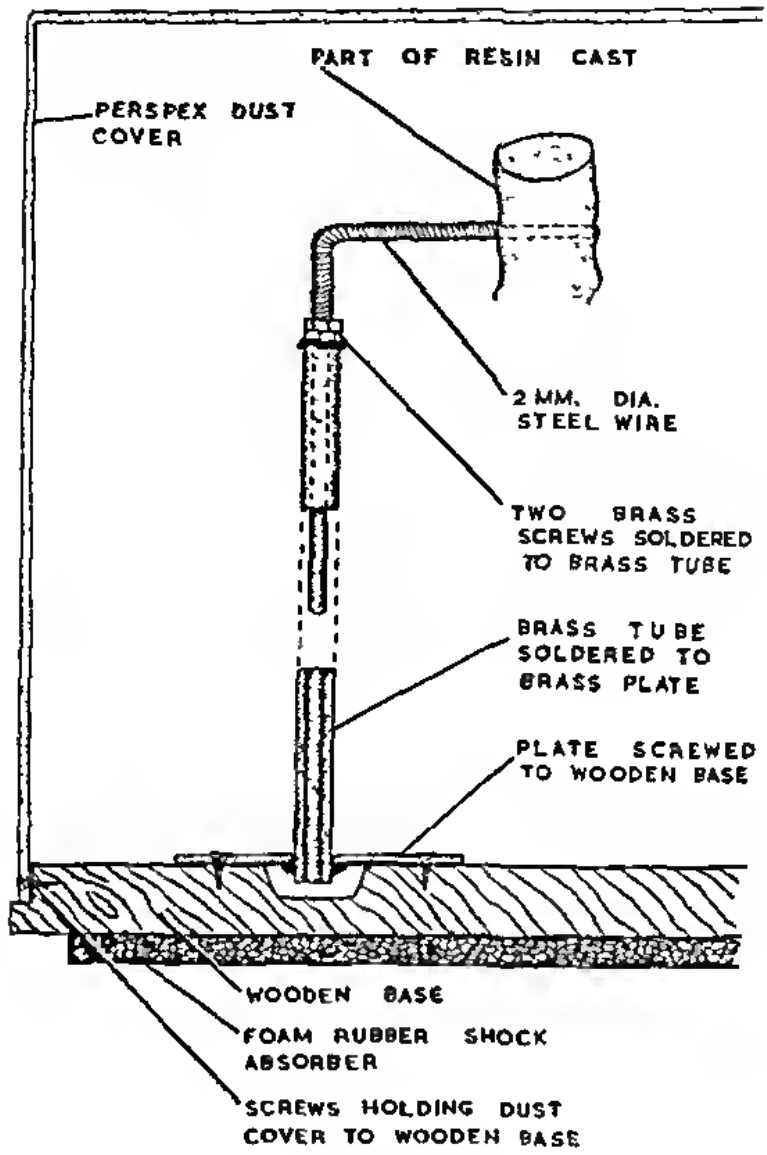

Fig 30

Diagram to show the method of mounting resin casts The piano wire is cemented into a hole drilled in the most convenient place in the resin cast, after one side of the end of the piano wire has been filed flat This precaution prevents the cast from rotating on the wire, if the cement becomes slightly loose The foam rubber sheet is stuck to the wooden base with Bostic adhesive No 252 (*see* Appendix)

of this method of mounting. While the mounting rod is being soldered into the brass tube, the minimum amount of heat necessary to melt the solder must be applied, as excessive heating of the mounting rod may damage the resin cement by which the rod is attached to the cast. To avoid the possibility of such damage it is advisable in many cases to solder the mounting rod into the brass tube *before* the rod is cemented into the cast. In this case a specially

shaped screwdriver may be required to tighten the screws by which the brass plate is fixed to the wooden base, as the resin cast usually gets in the way of the handle of an ordinary screwdriver. The Perspex dust cover should be screwed to the wooden base, to avoid an accident should the specimen be lifted by its dust cover, under the impression that this was firmly attached to the base, when in fact it was only held in position by friction. A sheet of foam rubber should be stuck on the base to act as a shock absorber.

Although slender branches of the resin are very brittle, the casts are not liable to suffer damage, if treated with normal care, even if the exhibits are frequently taken down from the museum shelf and handled, provided they are protected by the method described above.

3. CASTS FROM NEGATIVE MOULDS

The full practical details concerning the making of resin casts from negative moulds are given in Chapter 20. In the present chapter only the general considerations which determine the most suitable resin mixture for this type of work will be considered.

The resin mixture should have a comparatively low viscosity. This allows air bubbles, introduced when the accelerator is stirred in, to rise to the surface in a short time. It also makes it easier to get rid of any air which may be trapped in the mould when the resin is first run into it. Finally, as a mixture with a low viscosity flows more rapidly into the mould than a viscous one, it is possible to fill several moulds from one sample of resin before the latter gels. A mixture is recommended which contains 30-40 g. monomer C per 100 g. Marco resin 26 C.

For somewhat similar reasons, the mixture should have a relatively long working life. This period must allow sufficient time for all air bubbles, introduced when the accelerator is stirred in, to rise to the surface before the resin is run into the mould. It must also allow time, not only for the moulds to be filled, but for the manipulation of the moulds, followed by topping up with resin, which may be necessary to replace any air trapped within the moulds while they were being filled.

Usually a mixture with a working life of two hours is satisfactory for this work; but in the case of a comparatively bulky cast, *e.g.* one weighing more than 200 g., it is advisable to use a mixture with an even longer working life, to reduce the risk of the resin cast cracking as a result of overheating during polymerisation.

The somewhat brittle quality of the ordinary resin can be modified by

the addition of about 10 per cent Crystic resin 182, which acts as a plasticiser. But this material must not be used when a clear and as nearly as possible colourless cast is required, as it has a pale brown colour.

Pigment pastes should be used whenever possible in preference to powdered pigments for colouring resin which is to be used for filling moulds. If powdered pigments are used, the comparatively long period during which the resin is in the mould before it gels usually results in sedimentation of some of the larger particles of the powdered pigment, and this spoils the appearance of the finished cast.

For general work at a room temperature of 20°C, the following mixture is recommended :

Marco resin 26 C	100 g.
Monomer C	35 g.
Catalyst H C H	2 g.
Crystic resin 182	10 g.
Pigment paste as required to give the desired intensity of colour,	
Accelerator E	2 ml.

This mixture has a working life at 20°C of approximately two hours. If the room temperature is 24°C, the catalyst content should be halved. Provided that resin mixtures are used which allow an ample margin of time to complete the filling of the moulds before the resin gels, it is not necessary to make a test to determine the exact working life of the mixture used.

4. EMBEDDING SPECIMENS IN TRANSPARENT BLOCKS

Although wet specimens can be embedded in transparent blocks of resin, provided they are first surface-dried, this method of mounting cannot be recommended, as more satisfactory results are obtained, with less labour, by sewing the specimens to Perspex plates and mounting them in fluid in rectangular Perspex boxes by the method described in Chapter 9.

However, dry and fragile specimens may sometimes be mounted with advantage by embedding them in a solid block of resin, as this enables them to be roughly handled with little risk of serious damage. This method should only be used when it is specially desired to afford the specimen the maximum protection, as a rather foreshortened view is obtained of specimens mounted in this way.

Three special problems are encountered in this type of work. The first is a tendency of the resin to pull away from the embedded object, as a result of shrinkage which takes place during polymerisation. This produces mirror-like surfaces between the specimen and the resin in which it is embedded, which spoil the general appearance of the finished exhibit. It can be prevented by coating the specimen with Marco resin 26 C, and allowing this coat to set before the specimen is embedded.

The following formula is suitable at a room temperature of 20°C :

Marco resin 26 C	100 g.
Monomer C	25 g.
Catalyst H C H	4 g.
Accelerator E	2 ml.

Before coating the specimen with resin, a length of wire is cemented with resin cement into a hole drilled into the most convenient place. The wire not only serves as a handle with which to hold the specimen while it is being coated, but it later provides a convenient means of suspending it in the resin in which it is being embedded.

The specimen is dipped in the resin mixture, and the surplus is allowed to drain off. Then the specimen is rotated until the resin has gelled, so that it is covered by an even film. The surface of this film remains permanently tacky, and so the resin in which the specimen is embedded does not pull away as it would from a hard surface.

The second problem in block embedding is to avoid the block cracking as a result of excessive heat production during the heat-producing phase of polymerisation. This danger can be reduced in several ways. A mixture can be used which contains an unusually small percentage of catalyst and accelerator, and which may consequently take several days to gel. The heat is evolved by this type of mixture very much more slowly than from one which has a comparatively short working life. Consequently, if the resin is efficiently cooled during polymerisation, the reaction can be controlled, so that the resin never heats up more than a few degrees above room temperature. But as a substantial rise in temperature of the resin greatly accelerates the rate of polymerisation, it is essential to adjust the general conditions, especially when comparatively large blocks are being cast, so that the temperature of the resin never exceeds 25 C. The range of room temperatures at which this work can be most easily done lies between 18° and 20°C. Below 18°C the rate of polymerisation is retarded very greatly and somewhat unpredictably. Above

20°C the working temperature is dangerously near the maximum temperature to which the resin may be permitted to rise during polymerisation, without serious risk of the reaction getting out of control.

The third problem is to avoid the shrinkage, which takes place after the resin has gelled, from causing the transparent block to crack as a result of internal stresses set up by the resin sticking to the walls of the container in which it is polymerised. This danger is eliminated by using a comparatively flexible container and coating the inside with a separating medium. A sufficiently flexible rectangular container, which allows efficient cooling of the resin, can be constructed of 20 gauge tinned iron sheet. This can easily be cut with suitable shears. The container is made from three pieces of tinned sheet. One piece, twice folded, forms the bottom and two sides, while the other two sides are made from pieces which project beyond the bottom and sides of the container (see Fig. 31 A). This design facilitates both the fixing together of the three pieces of tinned sheet by soldering, and their subsequent removal from around the block of resin. It has the further advantage that by keeping the bottom of the container raised up, it allows more efficient cooling in a water bath than if an ordinary tin box were used.

The best method of holding a specimen in the resin so that it is correctly orientated is, in most cases, to suspend it by means of a wire, previously cemented to the specimen. The specimen is held in exactly the desired position by passing the wire through a hole drilled in a strip of Perspex, which is allowed to rest on two sides of the container. The wire is held in place by means of a lump of Plasticine, and the Perspex strip is prevented from moving by means of two pieces of Sellotape, which attach it to the outside of the container, as shown in Figure 31 A.

The alternative method of embedding a specimen in a block is to polymerise a layer of resin in the bottom of the container, and then lay the specimen on this, and pour in more resin until the specimen is immersed to a suitable depth. If the specimen floats in the resin, it is necessary to perform the embedding in three stages instead of two. But this method, though frequently recommended by many workers in the past, is unsatisfactory, except when the specimen is only to be viewed from above or below, as for instance if it consists of a slice or section. In lateral views of a block made by polymerising the resin in two or three layers, the refractive index of the resin in the region of each layer is substantially different from that of the rest of the block, so that the sites of the original layers are both conspicuous and unsightly in the finished block. The presence of a wire passing from the outside of the

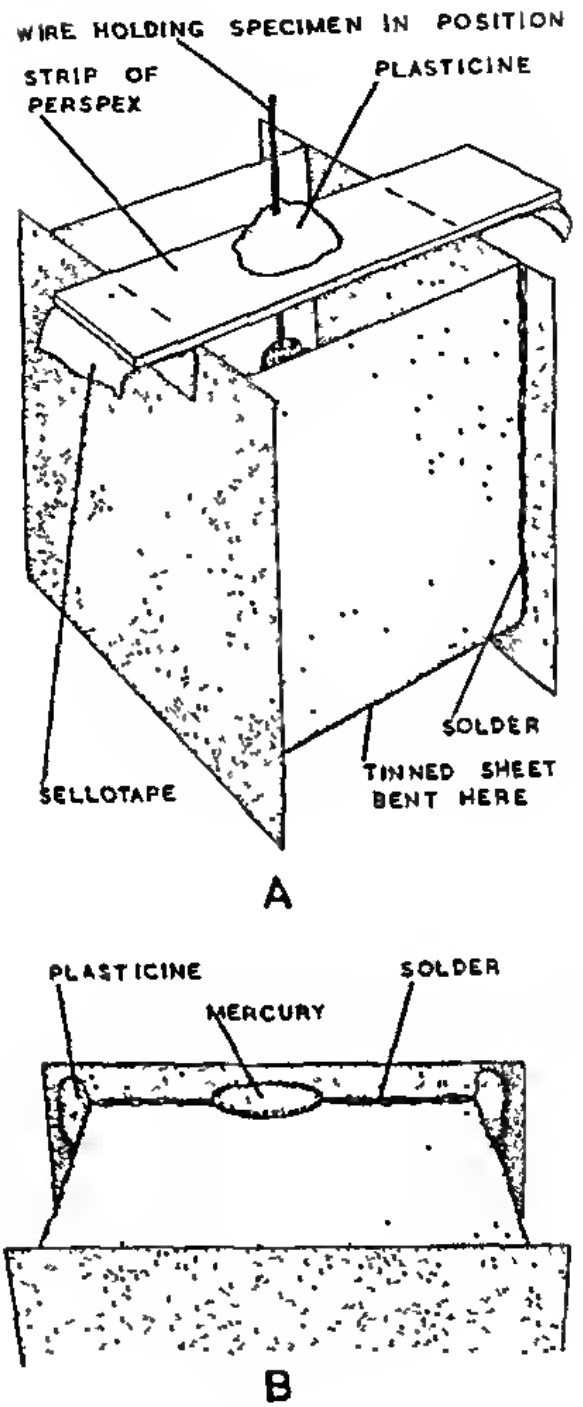

FIG. 31

A. Diagram of the type of container used to hold the resin, when a specimen is being embedded in a rectangular block of transparent resin. The figure shows the details of construction, and the method by which the specimen is suspended in the resin. B. Diagram to show how mercury is applied to the outside of the joints of the container to rot the solder.

finished block to the specimen is less displeasing to the eye than the presence of layers in the resin.

Before use the inside of the container is coated with a 20 per cent solution of shellac dissolved in industrial spirit. The shellac, which acts as a separating medium, must be allowed to dry thoroughly before the resin is poured into the container.

During the polymerisation of the resin, the container is placed in a large basin of cold water, to prevent overheating. In the case of a container larger than 3 x 4 inches in cross section, running water should be used to cool the resin, and it may also be necessary to place the container in ice water as soon as the resin begins to gel.

The following resin mixture is recommended for making blocks :

Marco resin 26 C	100 g.
Monomer C	20 g.
Catalyst H C H	0.3 g.
Accelerator E	0.2 ml.

At a working temperature of 20°C this mixture gels after a period between 80-100 hours in very subdued light. Moderately bright daylight substantially reduces the time the mixture takes to gel.

The container is left for three days in the water bath after the resin has gelled, in case there is any further evolution of heat. Then it is placed in an oven at 45°C for a further fourteen days, to complete polymerisation.

The tinned sheets are removed from around the block of resin by first rotting the solder with mercury. The container is held so that the solder along each of the joints in turn forms the bottom of a horizontal trough, the sides of which are provided by the tinned sheet, and the ends made by applying lumps of Plasticine to the tin (see Fig. 31 B). A little mercury is poured into the trough and rubbed into the solder of each joint for about half a minute with the tip of a finger. This work should be done over a large enamel meat tray, so that if any mercury is spilled it is not lost. By the time all the joints have been treated in this way, the first can easily be separated by gripping the projecting flange of the container with pliers and pulling it away from the resin block. The mercury is contaminated by the solder and so should not be mixed with pure mercury. It may, however, be kept in a separate bottle and will serve this purpose many times.

When embedding is done in layers, the danger of cracking which results from overheating of the resin is very much less than when the block is cast

in a single stage. Consequently a mixture with a shorter working life can be used, the exact constitution depending on the thickness of each layer.

If a layer of fairly large area is being cast, as for example 4 x 4 inches or more, the resin warps badly in the horizontal plane unless a rigid base is provided to which the resin adheres. A suitable stiffener may be provided by placing a piece of glass in the bottom of the container. The glass need only fit roughly, but should be cleaned in chromic acid before use to make it entirely grease-free, as this greatly facilitates its subsequent detachment from the resin block. The glass is removed by placing the hardened block in boiling water for about a minute and by cutting away the somewhat softened resin in which the glass is partly embedded. Then the glass can usually be removed fairly easily, either in one piece, or after cracking it with a hammer.

The method of trimming the block, smoothing the surfaces, and polishing them is described in Chapter 16.

Chapter 18

CASTS FROM THE LUNGS

1. INTRODUCTION

BEFORE satisfactory corrosion casts can be made from the lungs, some means must be devised by which the latter can be maintained in the expanded condition while the injection is being made, and during the subsequent period which elapses before the injection mass is fully hardened. Casts made from totally collapsed lungs would have little or no value.

The simplest way of achieving this object is to make the injection while the lungs are still in the unopened thorax but, although the lungs are not actually collapsed in the cadaver, they are by no means fully expanded. There are two further objections to this procedure. Very little manipulation and control of the conditions under which the injection is made are possible under these circumstances. And for many people, the necessary facilities are not available for doing this work while the lungs are in the cadaver. For the great majority of workers it is necessary to devise a technique by which lungs, removed at post mortem, can be expanded and injected in the laboratory.

Methods used in the past to expand lungs after removal from the body include suspending them in a vacuum chamber, and inflating them; but neither of these methods is really satisfactory. For the techniques described in this chapter, lungs removed at post mortem are expanded before the resin injection is made by filling them with a warm gelatine solution. The lungs are immersed in warm water, which not only prevents the gelatine from setting before the resin injection has been completed but, by supporting almost all their weight, avoids distortion due to the force of gravity. When expanded with gelatine under these conditions, the lungs naturally assume their anatomical form, and at the same time they resist strongly excessive over-expansion. But the gelatine diffuses so freely through the tissues of the lungs, and even through the pleura, that the resin injection easily displaces the gelatine from the bronchial tree.

As soon as the resin injection has been completed, the lungs are cooled, so that the gelatine solidifies. Hence the lungs are fixed indefinitely in the expanded form, while the injection mass hardens.

When Marco resin is used for vascular injections, some degree of over-injection invariably occurs, which necessitates pruning of the cast. But in the case of the bronchial tree, although the gelatine in the lungs retards the flow of the resin into the alveoli, certain special refinements of technique are necessary. Otherwise an almost solid cast of the whole lung may be produced, as a result of a large volume of resin entering the alveoli. It may be impossible to prune such a cast so that the main branches of the tree are displayed, without serious damage. This problem is not solved simply by limiting the amount of resin injected. For if the correct amount of resin is run in, either as a slow trickle, or several minutes before it solidifies, much of the resin gravitates into the alveoli of whatever part of the lungs is lowest during the injection, leaving the upper bronchi empty so that an incomplete cast is produced. This is avoided by three means :

1. By using a very viscous resin mixture.
2. By using an apparatus which allows even a viscous resin mixture to flow rapidly into the trachea.
3. By exceptionally careful timing of the injection.

But in spite of these precautions, casts of the bronchial tree made by the method described below always require extensive pruning. However, this rather tedious work is compensated for by the fact that, if the technique is skilfully applied, an almost perfect cast is invariably obtained.

If a cast of the pulmonary arteries and/or veins is made in conjunction with a cast of the bronchial tree, the only additional difficulty encountered is that involved in removing the macerated lung tissue from the cast. This may require dissection as well as washing. The labour of pruning is also greatly increased. A bronchial tree alone can be pruned in two days, but the pruning of a triple cast may take up to two weeks.

A cast of the bronchial arteries should only be made in conjunction with a cast of the bronchial tree, as the delicate cast of the arteries requires the tree to support it.

For these techniques the best results can only be obtained if healthy lungs, free from accidental cuts, are used. Although it is desirable to receive the lungs as soon after death as possible, as this facilitates the washing out of the bronchial tree and blood vessels, satisfactory results are usually obtained even if there has been a delay of several days, provided no decomposition has taken place.

For all lung casting, a suitable Perspex tray (see Fig. 32), shaped roughly like the posterior wall of an average-sized thoracic cavity, is required, to support the lungs in their correct position in relation to each other during the injection of the resin, and to facilitate their transfer without damage to the cast, from water to acid and vice versa.

FIG. 32

Perspex tray in which the lungs are placed during the injection of the resin, and by means of which they are subsequently lifted, when they are transferred to the acid bath

The tray is made in the following way. A model is made with modelling clay, similar to that shown in Figure 33A, which is actually a plaster positive mould made from a model. A negative mould (see Fig. 33B) is cast from the clay model, with Dentruset plaster. From the negative mould a plaster replica of the clay model is made. The walls of the positive mould are scraped away to a depth of ⅛ inch, to allow room for the Perspex sheet which is pressed into the required shape by means of the two plaster moulds. After the scraped surface of the positive mould has been smoothed with abrasive paper, both moulds are placed in an oven at about 45° C until they are quite dry and warmed right through. They are then impregnated with Marco resin 28 C, to strengthen them.

400 ml. of the following mixture are prepared :

Marco resin 28 C	100 g.
Monomer C	30 g.
Catalyst H C 11	1 g.
Accelerator E	1 ml.

The moulds are removed from the oven and placed in a large enamel meat

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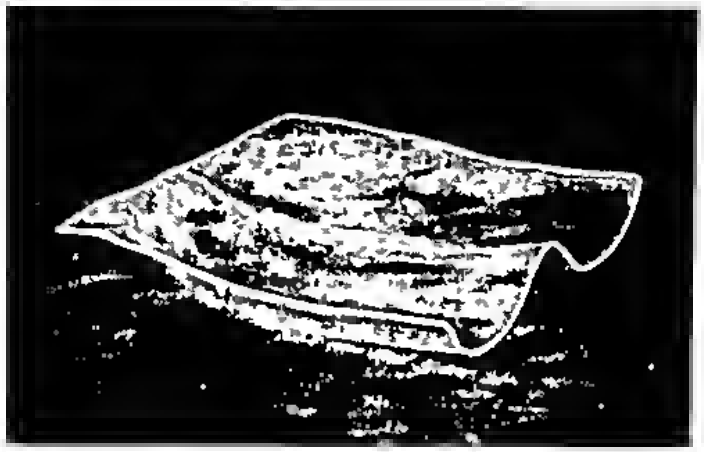

FIG 32

Perspex tray in which the lungs are placed during the injection of the resin, and by means of which they are subsequently lifted, when they are transferred to the acid bath

The tray is made in the following way. A model is made with modelling clay, similar to that shown in Figure 33A, which is actually a plaster positive mould made from a model. A negative mould (see Fig. 33B) is cast from the clay model, with Dentruset plaster. From the negative mould a plaster replica of the clay model is made. The walls of the positive mould are scraped away to a depth of ⅛ inch, to allow room for the Perspex sheet which is pressed into the required shape by means of the two plaster moulds. After the scraped surface of the positive mould has been smoothed with abrasive paper, both moulds are placed in an oven at about 45°C until they are quite dry and warmed right through. They are then impregnated with Marco resin 28 C, to strengthen them.

400 ml. of the following mixture are prepared :

Marco resin 28 C	100 g.
Monomer C	30 g.
Catalyst H C H	1 g.
Accelerator E	1 ml.

The moulds are removed from the oven and placed in a large enamel meat

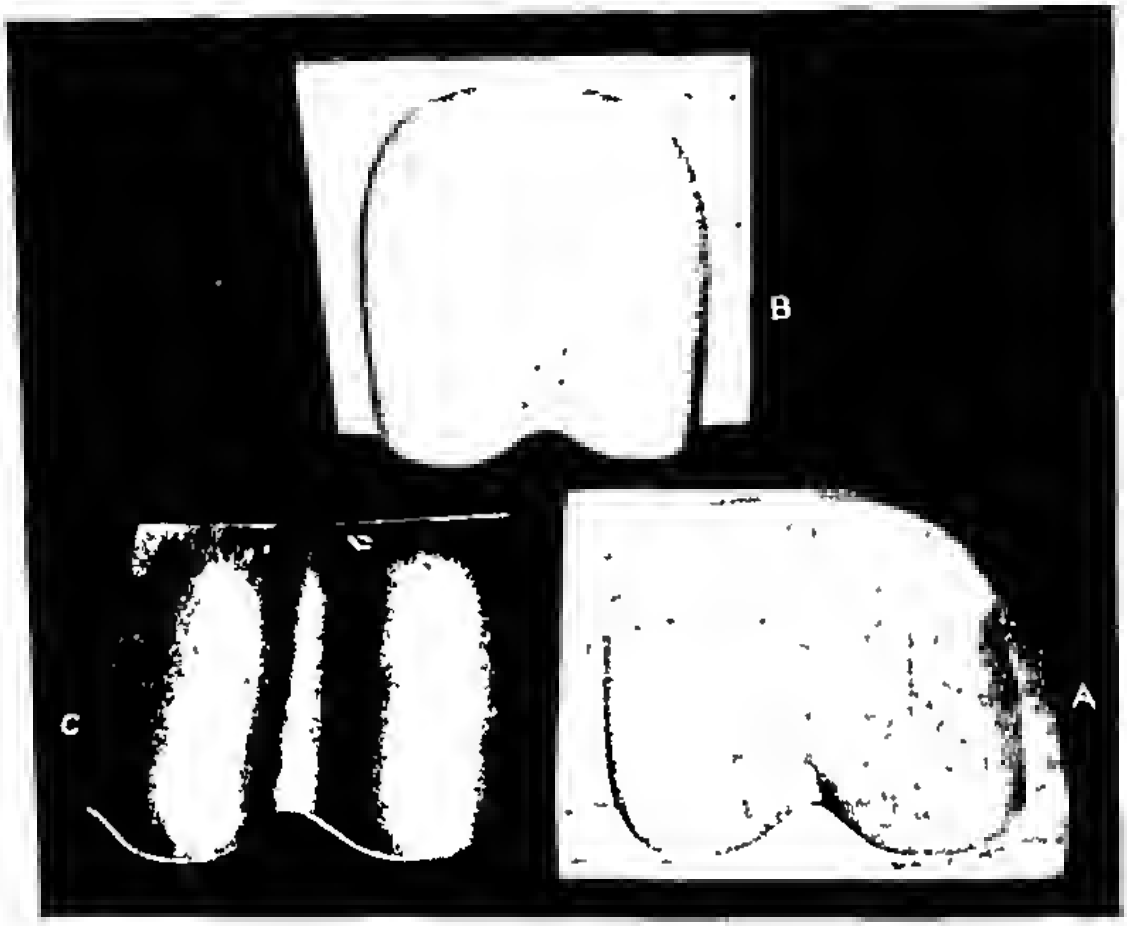

FIG. 33

The plaster moulds used for forming the Perspex lifting trays A is the positive mould, B the negative mould, and C a Perspex tray made by heating a sheet of Perspex until it is flabby, and pressing it between the two moulds

tray. The resin is applied very liberally with a medium-sized paint brush. As the moulds cool, the air within the porous plaster contracts, and this greatly assists the penetration of the resin. The resin is applied all over the surface of each mould, until no more is absorbed, or until the whole of the resin is used up. Any resin which remains on the surface of the moulds is removed with a wad of cotton wool soaked in acetone. The surface of the moulds after coating with resin should appear matt, not glossy.

Each mould is propped up on the edge of the meat tray until the resin is quite hard. The moulds should not be used until a week after impregnation, so that the resin is fully hardened.

Although the preparation of the moulds is somewhat laborious, any number of Perspex lifting trays can be made with them, with little additional labour. Those who require a Perspex lifting tray are therefore advised to contact some department where casts of the bronchial tree have already been made,

with a view to purchasing a lifting tray from someone who possesses the necessary moulds.

To make a Perspex lifting tray, a sheet of ⅛ inch thick Perspex, about an inch larger all round than the mould, is placed in a domestic oven, the temperature of which is between 150°-160°C. The Perspex is left in the oven until heated evenly right through, when it becomes quite flabby. To ensure that the sheet is heated evenly it may be necessary to change its position in the oven after ten minutes, and to leave it in for a further ten minutes. Excessive heating must be avoided as it causes the Perspex to blister.

When the Perspex sheet is quite flabby, it is removed from the oven and quickly placed on the positive mould, which rests on the floor. The negative mould is placed on top, and then the operator stands on the latter until the Perspex has cooled sufficiently to become rigid. Finally surplus Perspex is trimmed from the sides of the tray on the bandsaw.

2. THE BRONCHIAL TREE

(*a*) ADULT

A short length of Portex vinyl V.Y. standard tube, of such diameter that it fits easily, is tied into the trachea very firmly with fine string. The lungs are placed in the sink, which has previously been filled with cold water, and the cannula in the trachea is connected to the cold tap by means of rubber tubing. The tap is then adjusted so that there is a gentle flow of water into the trachea. The water expands the lungs and washes any mucous in the bronchial tree into the alveoli. The water then diffuses freely through the lung tissue, escaping partly via the cut ends of the pulmonary vessels and partly through the pleura. Thus most of the blood in the pulmonary vessels is also washed out. Washing is continued until the water escaping from the pulmonary veins is almost free of blood pigment. Usually washing must be continued for about an hour.

Next the lungs are deaerated. This is done by means of the apparatus shown in Figure 22 (p. 70) by alternately inflating the lungs with carbon dioxide from a cylinder, and evacuating them by means of a water vacuum pump. This treatment is repeated a dozen times and results in the residual air in the lungs being almost completely replaced by carbon dioxide. The carbon dioxide is removed by running a large volume of deaerated water through the lungs. The carbon dioxide dissolves in the water.

Next the lungs are disconnected from the apparatus used to deaerate them, and gently compressed to expel most of the water they contain. Then 5 litres of 70 per cent spirit are injected via the trachea, and the lungs are transferred to a tank of 70 per cent spirit. Before they are lifted out of the water in which the previous operations were carried out, the end of the cannula inserted into the trachea is closed by fitting a short length of rubber tubing, with a screw clamp attached to it, to prevent the entry of air.

The lungs may be left in spirit for several weeks without deterioration, but in this case a considerable quantity of spirit should be run into them at least once a week, to re-expand them. They may be injected with resin after a minimum of two days in the fixing tank, but the injection with resin of unfixed lungs is not advisable.

The day before the resin injection is made, the lungs are placed in a sink filled with cold water, and a large volume of deaerated water is run into them to wash out the spirit. It is necessary to deaerate the water used for washing out the spirit, as tap water is frequently supersaturated with air, which comes out of solution in the lungs if the water is run in directly from the tap.

A sufficient number of leaves of sheet bone gelatine, 120 bloom, to yield about 6 litres of gelatine solution when melted, are put to soak in cold water (as described on page 71). 28 lb. of ice should be ordered for use at the time when the resin injection is being made.

The lungs are left overnight immersed in cold water. The next day, the gelatine leaves are surface-dried and packed into 7 lb. Kilner jars. These are placed in a sink filled with hot water, to melt the gelatine. Lids must be placed on the jars to prevent a skin forming on the surface of the melted gelatine. The melting point of the gelatine is 28°C. It is used at 32°-33°C, and should not be heated many degrees above this temperature, as considerable delay may be caused while so large a volume is being cooled.

The temperature at which the gelatine is used is of great importance to the success of this technique. It is used at the lowest temperature which does not involve the risk of it setting while being injected into the lungs. If it is used at a higher temperature, it is not sufficiently viscous, and diffuses so rapidly out of the lungs, that it fails to maintain them fully expanded until the injection has been completed. Even when used in this viscous state, the gelatine is readily displaced from the bronchi by the resin.

600-700 ml. (according to the size of the lungs) of the following resin mixture are prepared:

Marco resin 26 C	100 g.
Monomer C	15 g.
Catalyst H C H	4 g.
Accelerator E	4 ml. (if room temperature is 20°C)

If the room temperature is 22°C only 3 ml. accelerator should be used; if 24°C only 2 ml. This technique should not be attempted if the room temperature is over 24°C. Whenever possible the working temperature should be 20°C.

A careful test is made to establish the working life of this resin mixture (see p. 113).

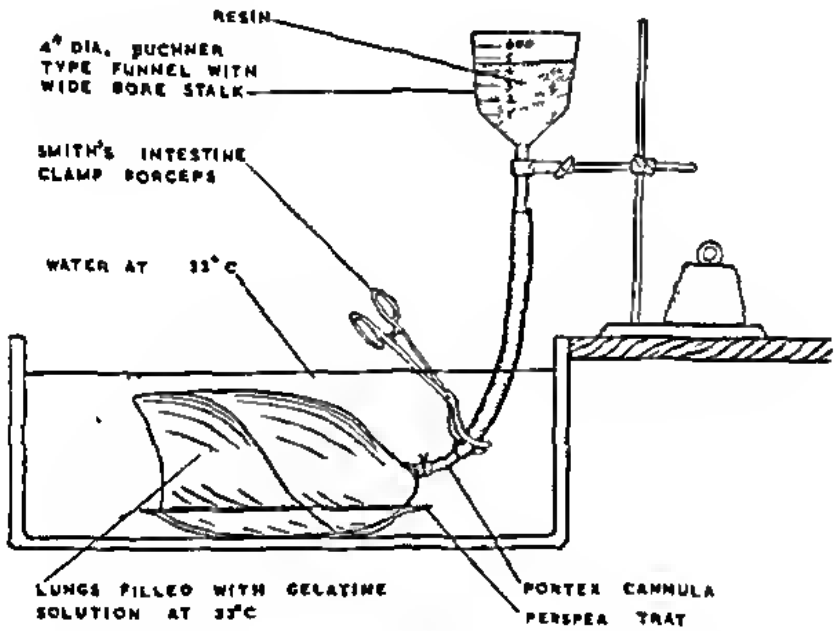

FIG 34

Apparatus for injecting the bronchial tree with resin. The injection is commenced by releasing the clamp forceps

The apparatus (see Fig. 34) from which the resin is injected consists of a four-inch diameter Buchner type glass funnel, which is connected to the cannula, previously tied into the trachea, by a length of rubber tube. The funnel, which is calibrated in units of 100 ml., is held about eight inches above the lungs by means of a retort stand and clamp. The rubber tubing must have an internal diameter of not less than ½ inch, as otherwise the viscous resin does not flow sufficiently rapidly to ensure a successful injection.

The lungs are prepared for the injection of the resin by raising the temperature of the water in which they are immersed, to 33°C, and by running

some deaerated water at this temperature through the trachea to warm the lung tissues. Then the lungs are completely filled with the gelatine solution, which is run in by gravity flow from a funnel connected to the cannula in the trachea by rubber tubing. Immediately before use the funnel and rubber tubing are warmed by immersion in hot water, to prevent the gelatine setting in the apparatus. The funnel and tubing used to run in the gelatine must not be used for the injection of the resin as the cold resin mixture, coming in contact with gelatine in the rubber tube, would cause the gelatine to solidify, so that the flow of the resin would be obstructed.

The rubber tubing of the apparatus from which the resin is to be run in is clamped about one inch from its lower end, before it is connected to the cannula in the trachea, to prevent water or gelatine from entering the part above the clamp. The part of the rubber tube below the clamp is immersed in water, so that gelatine solution in it is kept warm and thus prevented from setting.

Next, the accelerator is added to the resin mixture and stirred in for two minutes. The injection is commenced four minutes before the resin is expected to gel. The resin is poured into the injection apparatus one minute before the injection is started, and the rubber tube manipulated to dislodge any air which may be trapped in it. If the lungs appear to be excessively turgid, just before the injection of the resin is commenced the rubber tubing is disconnected from the cannula, so that a little of the gelatine can escape from the trachea by back flow into the water bath. Figure 34 shows the apparatus the moment before the resin injection is commenced.

To begin the injection the clamp is released. The resin flows rapidly into the lungs. For normal-size lungs 300 ml. resin are run in and then the clamp forceps are applied to stop the flow. In the case of small lungs, 250 ml. are run in, and in the case of exceptionally large ones, 350 ml.

The initial injection, which is completed in less than two minutes, fills all the main bronchi. The resin tends to gravitate to the bottom of the lungs, leaving some of the upper parts of the tree empty. To compensate for this, every 30 seconds the clamp is released, while about 20 ml. of resin flows in. The amount injected is indicated by the passage of tiny air bubbles down the long wide stalk of the injection funnel.

When it is observed that the descent of these bubbles is becoming *unmistakably* slower, the resin is allowed to flow in continuously, as it is on the point of gelling. If there is any one secret of success in getting a really perfect cast of the bronchial tree, it is to ensure that resin is actually flowing

into the trachea at the moment when it gels. This ensures that the bronchial tree is completely full of resin.

The lungs are now arranged on the Perspex tray, and the position of the trachea is adjusted, so that the two lungs and the trachea are all in their correct relative positions. Then a large amount of ice is added to the water to cool it as rapidly as possible, so that the gelatine sets and holds the lungs firmly in the expanded form during the comparatively long period required for the resin to become fully hardened.

About half an hour after the resin has gelled, the rubber tubing and resin within it are cut through with a large pair of scissors. The rubber tubing is disconnected from the glass funnel. The resin, which has the consistency of hard cheese at this stage, breaks easily. The glass funnel is boiled in water containing about 1 per cent Homacol or other liquid toilet soap, for an hour. When the water has cooled to room temperature, the resin

remaining in the funnel can be easily removed. The resin in the rubber tube can be expelled and the tube cleaned as described on page 107.

The lungs are left immersed in cold water for six days while the resin attains full hardness. Figure 35 shows them lifted out of the water on the Perspex tray, during this period. Then for forty-eight hours they are immersed

FIG. 36

Cast made from the lungs shown in Fig. 35, after the tissues had been macerated in concentrated hydrochloric acid, and washed away with tap water. × ½.

in running water at between 35°-40°C, to melt and remove as much as possible of the gelatine. The temperature of the water must not exceed 40°C, as it may otherwise cause shrinkage of the tissues, and consequent distortion of the cast. The lungs must be thoroughly cooled in cold water before they are transferred to the acid bath, as otherwise the resin cast may be badly warped. Although it is not essential to wash out the gelatine first, this treatment reduces the period during which the lungs have to remain in the acid, and it also reduces the contamination of the acid, so that it can be used several times.

The lungs are next immersed in a bath of concentrated hydrochloric acid. A suitable bath is provided by pouring about 7 Winchester quarts of

acid into a large glass accumulator jar. When the lungs are first placed in the acid they float. They should be gently pressed down from time to time until they sink, to ensure that the part above the acid is macerated as rapidly as the rest of the tissues. Maceration is complete in one to three days, according to

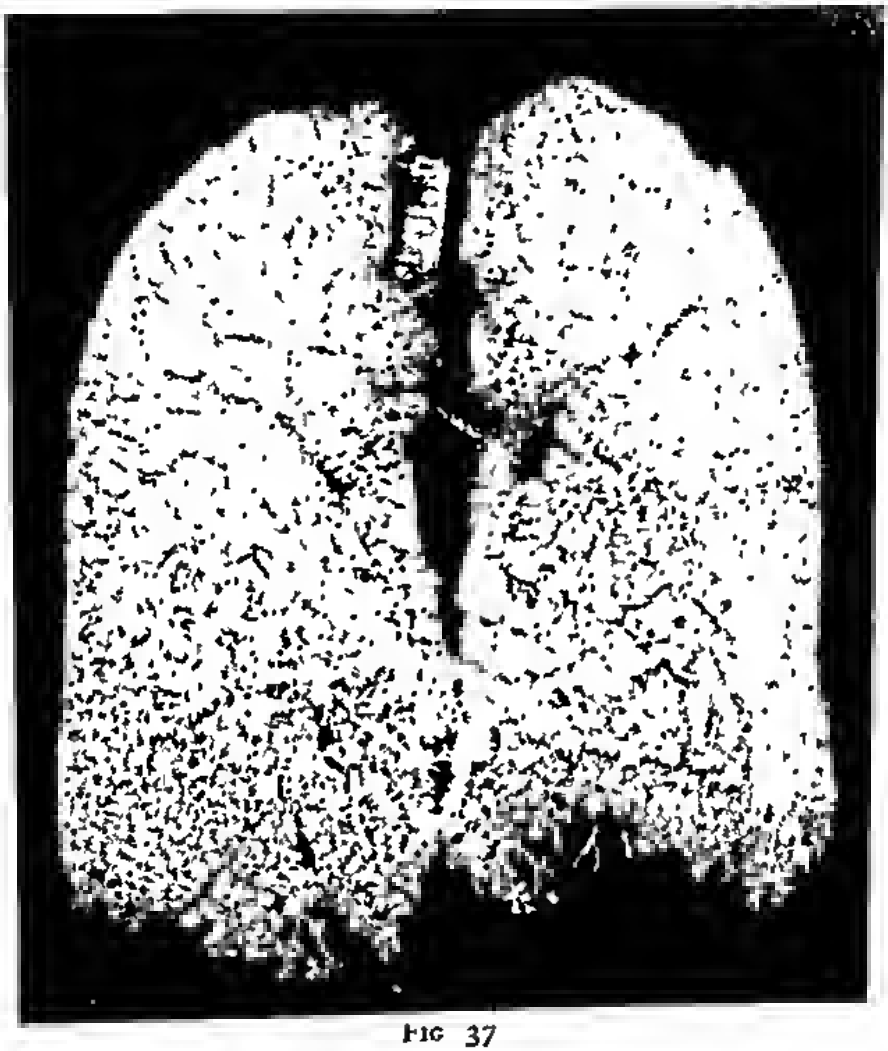

FIG 37

Unpruned cast of a bronchial tree, made by running in 350 ml resin instead of 250 ml as was injected initially in the preparation of the cast shown in Fig. 36 Note that the alveoli have been more completely filled with resin in this cast than in the one shown in Fig 36. × ⅓

whether the acid is fresh, or has been used previously. When maceration takes longer than three days it is time to use fresh acid.

The full details concerning the washing, pruning, repairing, cleaning and mounting of the cast are given in Chapter 17. Figure 36 shows the unpruned cast, prepared from the lungs shown in Figure 35, after the macerated tissue had been washed away. In this specimen the initial injection was limited to 250 ml. although the lungs were of normal size, as this cast was to be mounted unpruned. Consequently it was particularly desired to avoid

excessive filling of the alveoli. Figure 37 shows an unpruned cast of lungs of similar size, in which the initial injection was 350 ml. The alveoli of this specimen have been much more completely filled with resin.

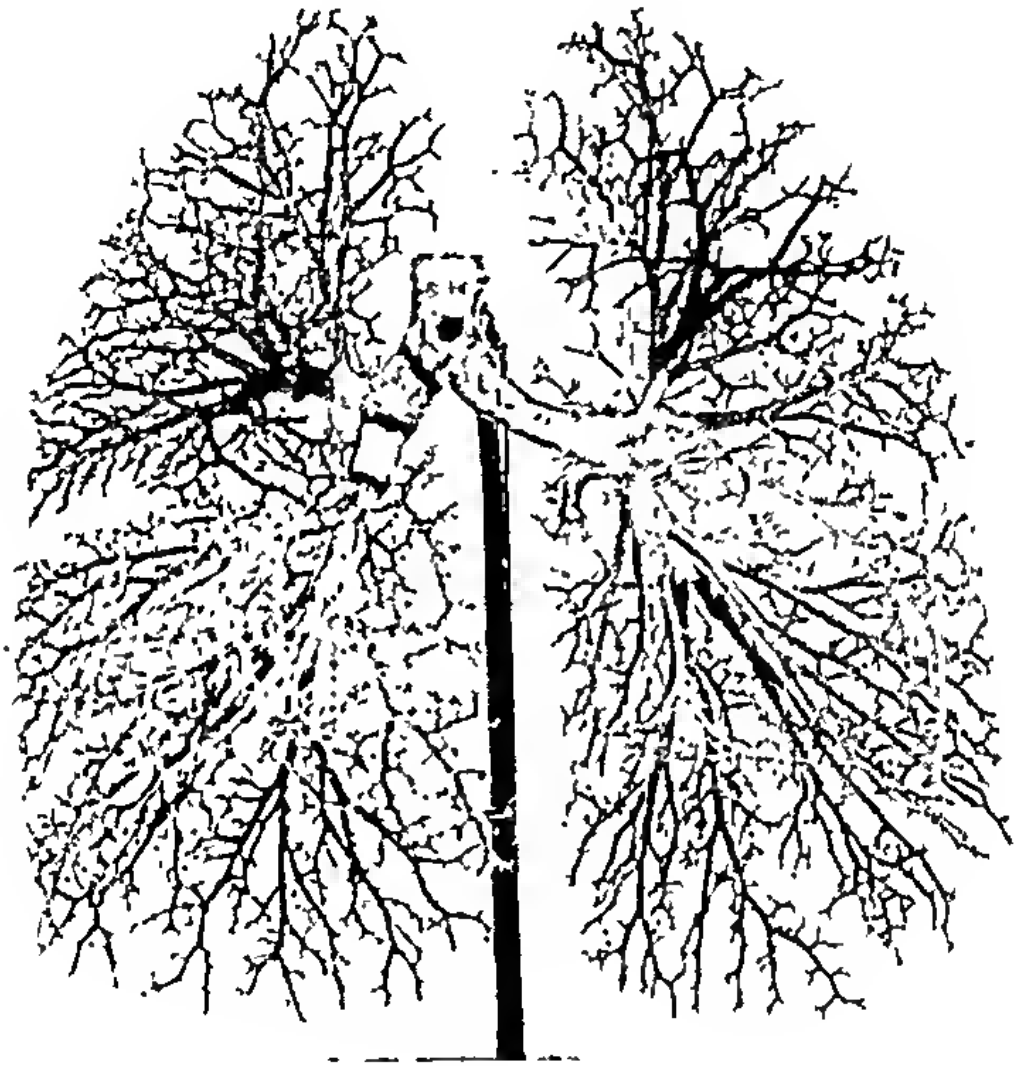

FIG. 38

Cast of a bronchial tree after pruning has been completed. The principal branches have been painted with cellulose paints. × 4/9

The main branches of the pruned tree can be differentiated by painting them with a quick-drying cellulose paint. Naylor's Brushing Belco (see Appendix) is recommended for this work. It is available in quarter-pint tins, and can be applied with a small sable water-colour brush. It dries in less than half an hour, leaving a glossy surface. The brushes are cleaned in acetone after use. Figure 38 shows a cast painted in this way.

(*b*) INFANT

Certain modifications of the technique described above are necessary when casts are made from the lungs of still-born or very young babies.

To avoid excessive hardening of the tissues, the lungs are fixed in 50 per cent spirit instead of the usual 70 per cent and injected with resin after not more than forty-eight hours fixation.

The gelatine is run in at 40°C, instead of at 33°C, to avoid the risk of it setting in the apparatus from which it is injected.

A less viscous resin mixture is also required. The following formula is recommended for the lungs of a fifteen-day old child at room temperature of 20°C :

Marco resin 26 C	100 g.
Monomer C	20 g.
Catalyst H C H	4 g.
Accelerator E	4 ml.

As it is necessary to observe accurately the quite small volume of resin which is injected, the resin reservoir is made from a short length of glass tube of internal diameter 2 cm. The lower end of the tube is drawn out to fit into the comparatively slender rubber tube by which the glass reservoir is connected to the cannula in the trachea. A 1 cm. fall in the level of the resin in the tube represents the injection of about 3 ml. resin. An initial injection of about 15 ml. resin should be made in the case of the lungs of a fifteen-day old child.

In the case of an older child, the modifications of technique are adjusted somewhere between those for adult and infant lungs, the exact details of the modifications depending on the age.

The pruning of the cast of the bronchial tree of a fifteen-day old child is most easily accomplished by holding the cast immersed in tepid water, and gently pulling away the surplus resin with iris forceps (see Fig. 9, p. 29). In tepid water the cast is quite flexible, but when dry and cold it is exceedingly brittle. In spite of this, such specimens, when properly mounted, can be handled without any risk of damage if they are treated with reasonable care. Perfect casts can be made from the tiny lungs, showing all the main branches characteristic of the adult tree. The casts are however too delicate for the branches to be painted, without risk of serious damage. A particular advantage of making casts of the bronchial tree of infants is that the tree can be pruned in half a day, instead of the minimum period of two days required for

the pruning of a cast of the tree of adult lungs. Figure 39 shows a cast of the bronchial tree of a fifteen-day old child.

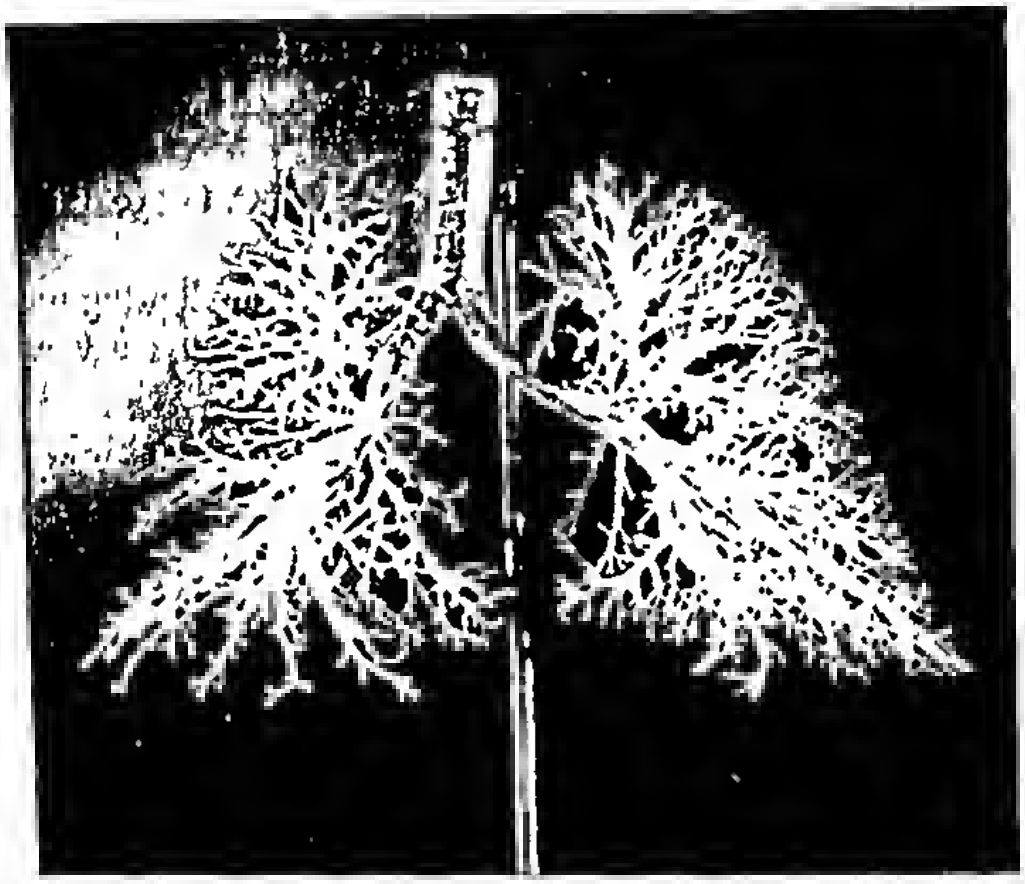

FIG 39

Cast of the bronchial tree of a 15 day old child (natural size).

3. THE PULMONARY VESSELS

If the pulmonary arteries are to be injected, at least an inch of the pulmonary trunk must be left attached to the lungs when the heart is removed, so that a length of Portex tube can be tied securely into it. In the case of the pulmonary veins, the whole of the left atrium and the adjacent part of the left ventricle must remain, so that the cannula can be inserted through the mitral valve, and tied securely into position. Special care is needed when the cannula is fixed into the left atrium. A finger is inserted through the mitral valve. Then a curved needle, threaded with linen carpet thread, is passed through the muscle of the left ventricle around the valve. If the needle comes out of the muscle at any point on the inside, the finger feels it, and so the needle can be partially withdrawn and its position corrected. Then a length of Portex vinyl V.Y. standard tube, of such diameter that it fits rather firmly

into the atrium, is inserted and tied in position. Unless the thread used to tie the cannula in position passes only through the muscle, serious leakage may result via the mitral valve when the resin injection is being made.

After most of the blood has been washed out of the vessels by the water run into the trachea, deaerated water is run directly first into the pulmonary trunk and then into the left atrium (if the veins are also to be injected). The success of the injection with resin of the pulmonary vessels depends on the latter being completely free from blood clots, so the washing must be very thorough. While the lungs remain in the spirit tank, whenever the bronchi are flushed out with spirit, the arteries and veins are treated in the same way.

When the spirit is being washed out of the lungs, on the day before the resin injection is to be made, water is run into the vessels as well as into the trachea.

The same formula is used for the resin mixture used to fill the pulmonary arteries and veins as that used to fill the bronchial tree. 500 ml. resin is required in each case. The resin used to fill the arteries is coloured by adding either 1 g. red lake pigment powder M. 11, or 2 g. red lake pigment paste B. 214 per 100 g. resin. In the case of the veins, either 1 g. blue pigment powder M. 21, or 2 g. blue pigment paste B. 266 are added.

The injection apparatus used to fill the pulmonary veins and/or arteries is identical with that used to fill the bronchial tree. The apparatus must be adjusted with special care to ensure that the rubber tubing which connects the glass funnels containing the coloured resin to the cannulae inserted into the vessels, does not cause the cannulae to be pressed against the lungs. Even slight pressure on one of the main bronchi can almost completely impede the flow of resin into the corresponding part of the lung. The position of the funnels from which the pulmonary vessels are filled with resin must be so adjusted that the lungs are almost lifted off the Perspex tray on which they rest.

The injection of the pulmonary vessels is made simultaneously with that of the bronchial tree, the coloured resin being run in from the same height in each case. When the vessels are full, the resin almost ceases to flow. In normal-size lungs this happens after approximately 200 ml. have been run in. At this point the rubber tubing is clamped to reduce the tendency for excessive injection and more particularly, in the case of the pulmonary veins, the tendency for the resin to soak into their walls. Whenever the bronchial tree is topped up, the clamps controlling the flow of the coloured resin are also released for a few seconds to ensure that the vessels are completely full of resin when it gels.

In the case of a triple cast, the macerated tissue always has to be dissected away, as it cannot be removed by washing alone. The finished cast is sprayed with Marco 28 C resin mixture by the method described on p. 123 to bring out to the best advantage the natural colours of the resin.

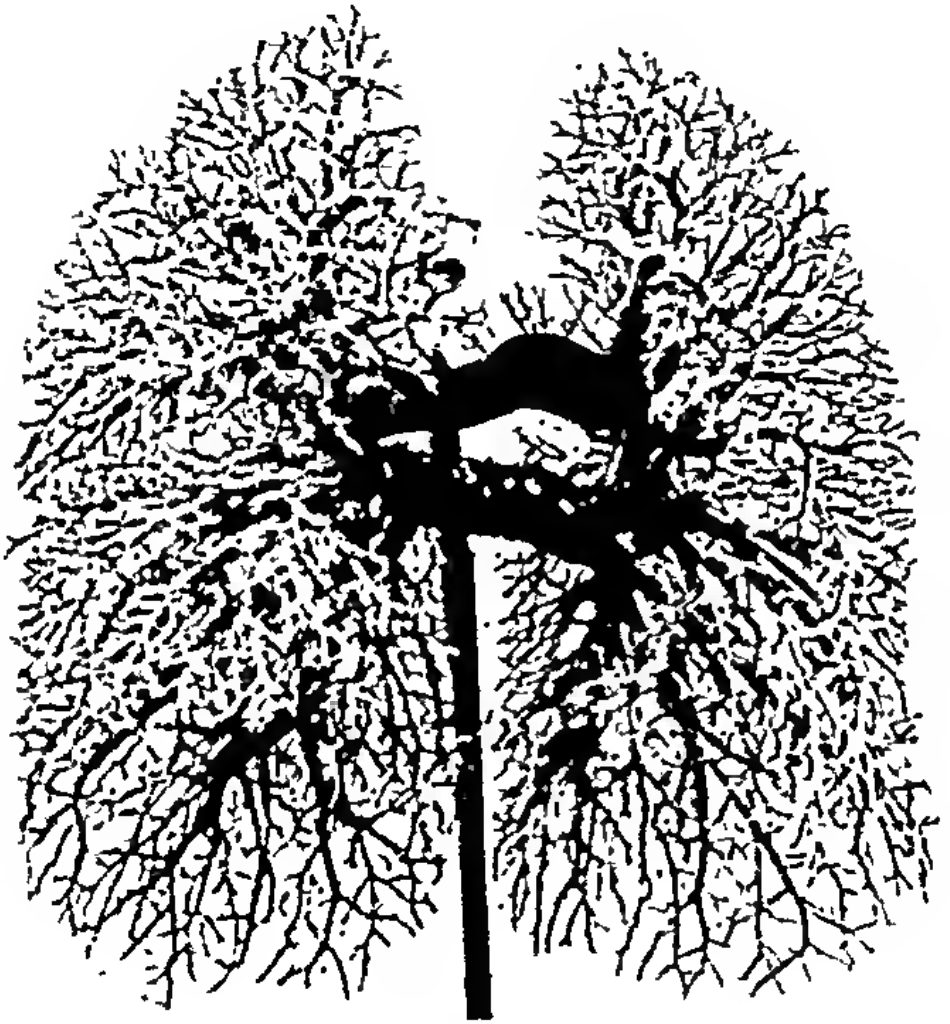

FIG. 40
Cast of the bronchial tree, together with casts of the pulmonary arteries and veins × 4/9

The frontispiece is an illustration of a cast of the bronchial tree with the pulmonary arteries; Figure 40 shows a cast of the bronchial tree together with casts of the pulmonary arteries and veins.

4. THE BRONCHIAL ARTERIES

If a cast is to be made of the bronchial tree and bronchial arteries, lungs are required with both the ascending and the upper three inches of the

descending aorta in situ, as the injection of the bronchial arteries is made via the aorta. The aorta must be carefully dissected away from the mediastinum, together with the stumps of the intercostal vessels. If it is dragged away, there is a risk that the intercostal vessels may break off at their point of origin from the aorta, with the loss of any bronchial twigs that may arise from them.

Short lengths of Portex vinyl V.Y. standard tube are tied into each of the cut ends of the aorta. A short length of rubber tubing is tied to the Portex tube in the end of the ascending aorta, and a screw clamp is attached so that this end can be closed when necessary. Except for the bronchial arteries all vessels given off by the aorta are ligatured. A cannula is also tied into the pulmonary artery.

After the lungs have been washed out in the usual way, by running tap water into the trachea, deaerated water is run into the pulmonary artery. As some of this escapes via the bronchial arteries, most of the blood remaining in the latter is also washed out. Next the rubber tubing attached to the cut end of the ascending aorta is closed, and deaerated water is run into the aorta by means of the cannula attached to the other end, to complete the washing out of the bronchial arteries. While water is being injected into the aorta at a moderate pressure, the lungs are partly raised from the water in which they are floating, so that the posterior wall of the aorta is above the water. A jet of water escaping indicates the presence of any unligatured intercostal artery, which is then tied.

A resin mixture of relatively low viscosity is required to fill the bronchial arteries. In order to reduce the inhibition of setting by water to the minimum, a mixture with an exceptionally short working life is used. Although only a very small volume of resin is required to fill the aorta and bronchial arteries, 400 ml. should be prepared, in order to have sufficient to make the usual test to establish the working life, and to provide enough to fill the injection apparatus, and also to allow for loss of resin during manipulations involved in making the injection.

The following formula is recommended:

Marco resin 26 C	100 g.
Monomer C	30 g
Catalyst H C H	6 g.
Red lake pigment powder M. 11	1 g.
Accelerator E	6 ml.

This mixture has a working life of approximately eleven minutes at 20°C. It is injected by means of the Standard injection apparatus (see Fig. 29, and description in text, p. 115).

Figure 41 shows the apparatus immediately before the injection is commenced. The injection is made in the following way. As soon as the accelerator has been incorporated into the red resin mixture, it is poured into

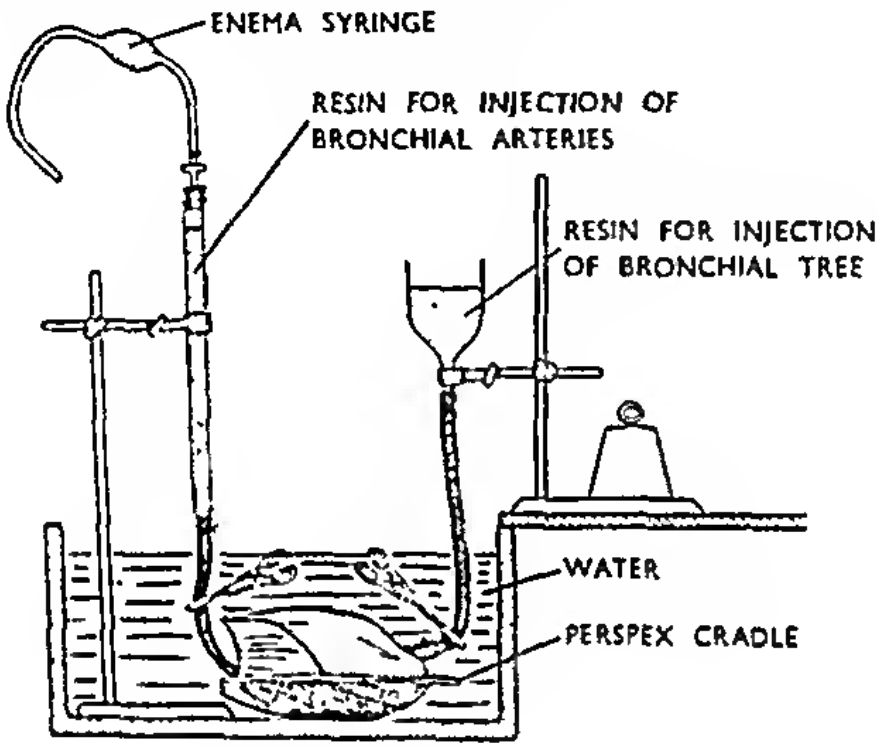

FIG. 41

Apparatus for the injection of the bronchial tree and bronchial arteries, at the moment before the injection is commenced. Fig. 29 (p. 115) shows more fully the details of the Standard apparatus used to fill the bronchial arteries. Fig. 34 shows in greater detail the apparatus used to fill the bronchial tree.

the Standard apparatus, as there is no time to waste if the injection is to be completed before it gels. After pinching the rubber tubing which connects the Standard apparatus to the cannula in the cut end of the descending aorta to dislodge any air trapped in it, the injection is commenced with the other end of the aorta open. This allows water in the aorta to be rapidly displaced by resin. When resin begins to escape via the end of the ascending aorta, the clamp which controls the opening at this end is closed. Next the resin level in the Standard apparatus is topped up and a considerable injection pressure is exerted by means of the enema syringe. An assistant applies this pressure, while the other worker feels with two fingers the turgidity of the aorta, to give

warning before the injection pressure becomes dangerously high. The injection of the bronchial tree is made simultaneously, in the normal way.

The day after the injection, the lungs are removed on their Perspex tray

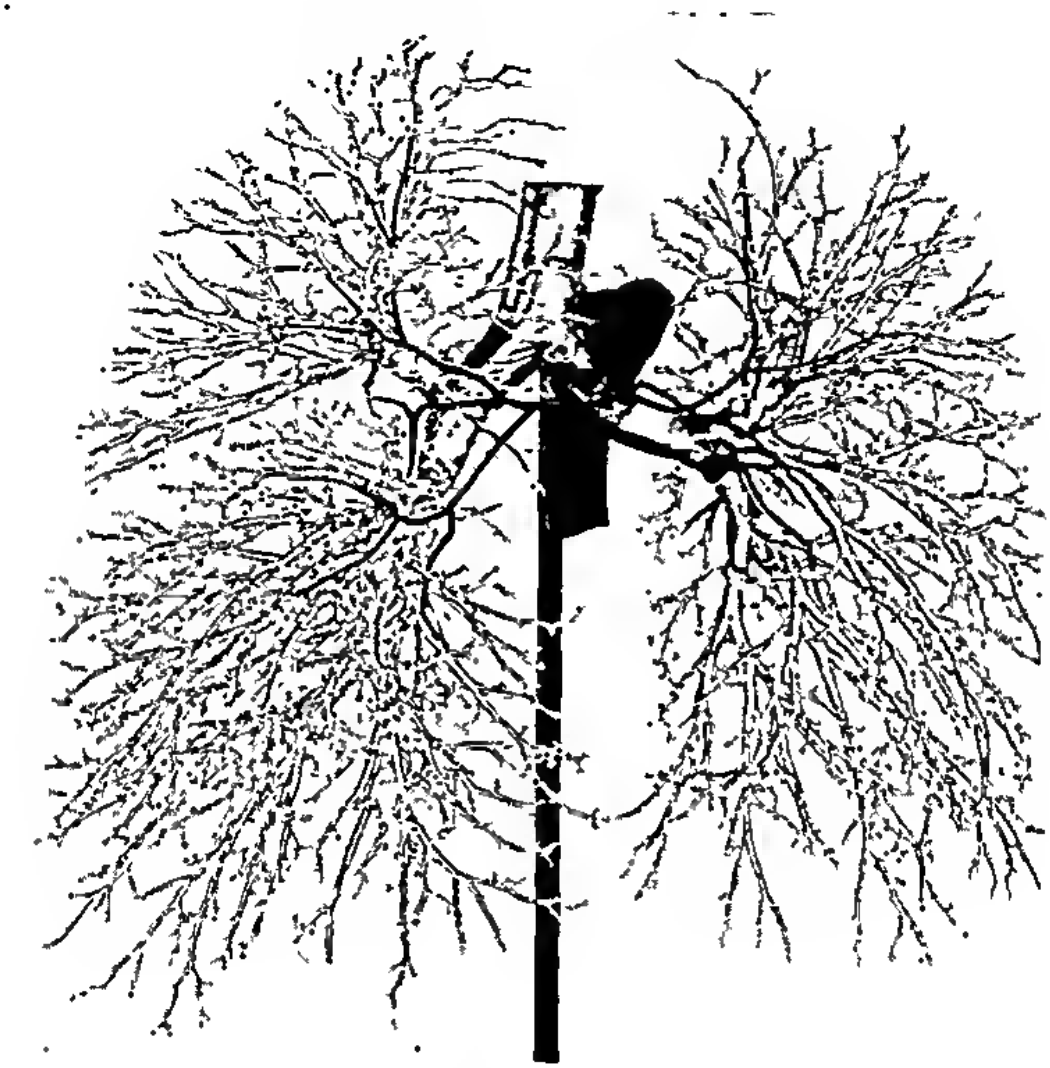

Fig 42

Cast of the bronchial tree together with the bronchial arteries × ½

from the water bath, and the part of the wall of the aorta which lies closest to the trachea, and the wall of the trachea next to it, are dissected away. A suitably shaped piece of resin is then cemented into the gap between the cast of the trachea and the aorta, so that the cast of the aorta is firmly fixed to the cast of the trachea. Great care is needed while this work is being done to avoid breaking the cast of the bronchial arteries.

After the lungs have been macerated in acid, the subsequent washing away of the macerated tissue must be done with special care, as the delicate cast of the bronchial arteries, although lying against the cast of the branches

of the bronchial tree, has no actual attachment to it, and its finer branches are easily displaced.

Only cold water is used for washing, as warm water makes the very slender cast of the bronchial arteries too flexible. Any arterial branches accidentally displaced during washing can be replaced while the cast is wet and consequently flexible. The cast of the bronchial arteries must not be touched when dry, as the slender filaments of resin become exceedingly brittle.

The two ends of the aorta are trimmed with burrs attached to the flexible arm of a dental lathe.

After the cast has been washed, pruned and cleaned, it is immersed in warm water. This makes the cast of the bronchial arteries sufficiently flexible for final adjustment to be made of the position of any branches which are displaced.

When quite dry the cast is sprayed with a Marco resin 28 C mixture, by the method described on page 123. This resin cements the finer branches of the cast of the bronchial arteries to the branches of the bronchial tree. Figure 42 shows a cast of the bronchial tree and bronchial arteries, the latter coloured red.

This technique should not be attempted by anyone who is not sufficiently experienced to be confident of obtaining invariably a perfect cast of the bronchial tree alone.

Chapter 19

CASTS FROM THE HEART

1. THE CAVITIES AND BLOOD VESSELS

THIS technique provides a method for demonstrating the principal branches of the coronary arteries and the larger cardiac veins. At the same time a cast of the cavities of the heart is produced. Although a low injection pressure is used, the thin-walled cavities of the atria and the right ventricle are somewhat distended, so that the casts of these cavities are slightly larger than the cavities were in life. But, in spite of this defect, the cast of the cavities serves the useful purpose of making it easy to visualise the form of the heart which contained them, and in this way increases the value of the cast of the vessels.

A fresh post-mortem heart, to which the lungs and ascending aorta are attached, is required for this technique. The lungs are carefully dissected away from the heart, so that about an inch of each of the four pulmonary veins remains attached to the left atrium. Short lengths of Portex polythene (or if sizes larger than 7.5 mm. diameter are required, Portex standard vinyl V.Y.) tubing are tied into the two left pulmonary veins, the left pulmonary artery, the superior and inferior venae cavae, and the aorta. Short lengths of rubber tubing with adjustable screw clamps attached are fitted over all the cannulae except those into one of the left pulmonary veins and the inferior vena cava, so that the openings from the cannulae can be controlled as the requirements of this technique demand. The vessels into which no cannulae have been tied are ligatured. Figure 43 shows a diagram of the heart with the tubes attached.

The first step is to wash out the blood both from the cavities and the vessels of the heart as completely as possible. In order to avoid the risk of air getting into some of the vessels, the heart is first immersed in water and manipulated in such a way as to remove any air already in the cavities, before the blood is washed out. The heart is kept immersed in water during all subsequent manipulations.

First a large volume of deaerated water is run into the left atrium via the cannula in one of the left pulmonary veins. The water is allowed to escape

via the other left pulmonary vein, until all the blood in the left atrium has been removed. Then further escape of water via the pulmonary vein is prevented by closing the screw clamp. The clamp at the end of the aorta is now opened, and more water is run into the left atrium. This flows into the left ventricle and escapes via the aorta. Finally the opening at the end of the aorta is closed, so that the water flows into the coronary arteries and escapes via the cardiac veins. Then the right side of the heart is washed out in a similar way.

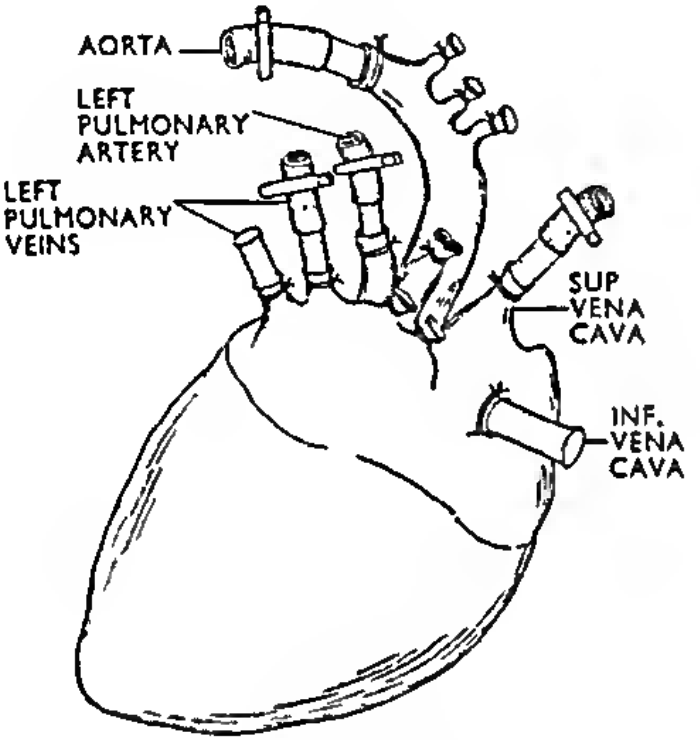

FIG 43

Heart after cannulae have been attached. Two of these are used to inject the resin into the two sides of the heart; the other four provide escape holes from the four cavities, to allow water to escape when the cavities are filled with resin. The openings of the escape holes are controlled by short lengths of rubber tubing and screw clamps

The heart is fixed by filling the cavities with 5 per cent formalin and placing it in a tank of 5 per cent formalin for about four days. Each day some formalin is injected by means of an enema syringe into the left pulmonary vein, with all clamps closed, and then into the inferior vena cava, so that the heart is slightly distended and fixative flows into the vessels. This ensures that the walls of the vessels are slightly stretched and also that any traces of blood remaining after the preliminary washing are removed.

The resin is injected fairly soon after the heart has been placed in the formalin tank, as excessive hardening by the fixative may impede the flow of the resin into the vessels. The injection of resin into the right side of the heart is made via the inferior vena cava, and of the left via one of the left pulmonary veins. The clamps controlling escape of fluid from the four chambers of the heart are slightly opened before the injection is commenced.

Figure 44 shows the apparatus used. The resin is injected by gravity flow from funnels held about 6 inches above the heart, and the apparatus is adjusted so that the heart is suspended in the water.

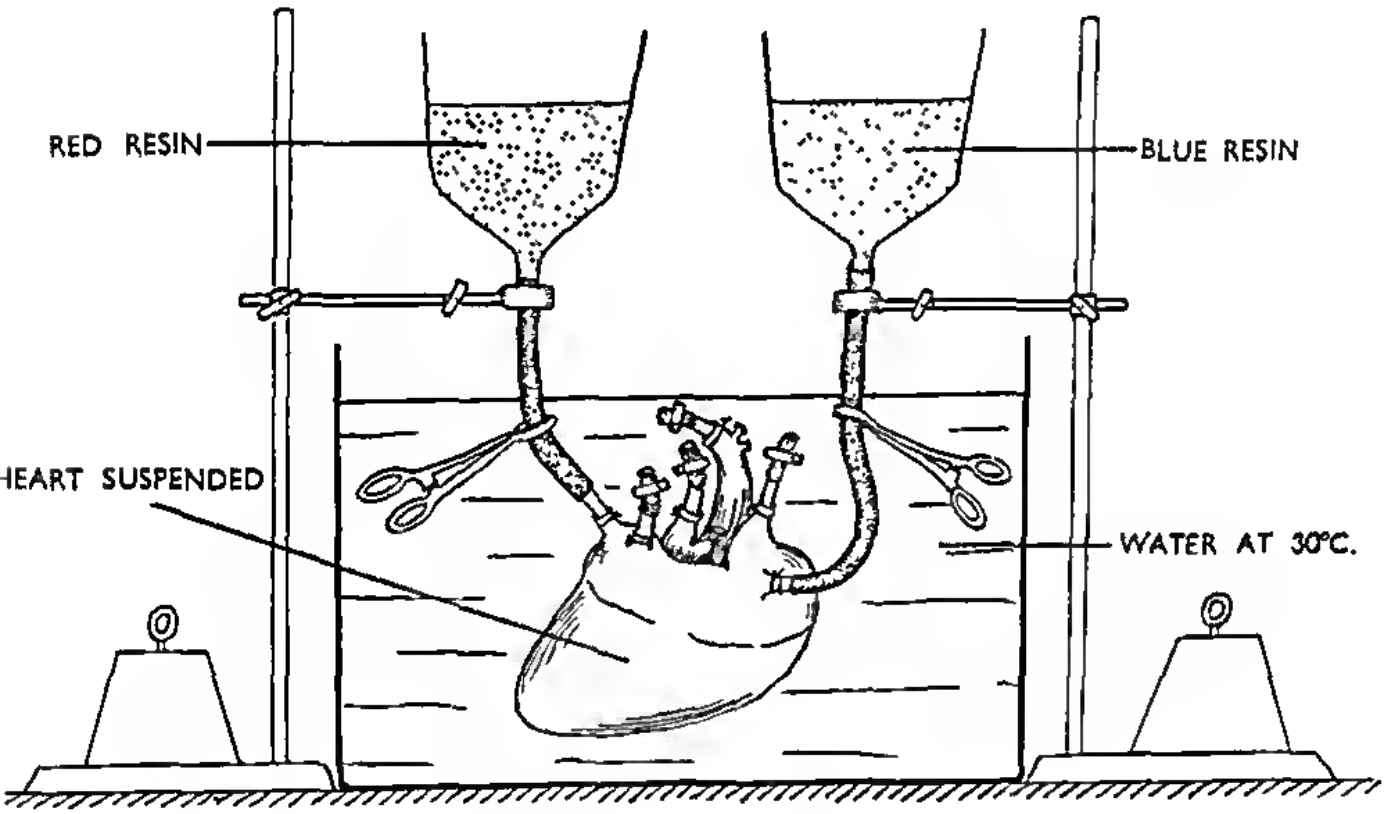

FIG 44

Apparatus for the injection of the resin

The particular problem involved in this technique is related to the fact that the same resin must be used to fill rather bulky cavities and also slender vessels. To fill the latter a mixture with a very short working life is most suitable, to reduce to a minimum the inhibition of setting. Such a mixture would almost certainly overheat and crack if the ventricles and atria were filled with it. A compromise must therefore be made between the mixture most suitable to fill the vessels and a safe mixture to fill the cavities. The most suitable resin mixture is the one with the shortest working life which does

not overheat and crack in the cavities. The exact formula of this mixture depends on the size of the heart. The formula given below is suitable for a heart of average size.

Two lots of 500 ml. of the following resin mixture are prepared :

Marco resin 26 C	100 g.
Monomer C	15 g.
Catalyst H C H	4 g.
Red lake pigment M. 11	
or blue pigment M. 21	1 g.
Accelerator E	4 ml.

If preferred pigment pastes red Crystie B. 214 and blue B. 266 may be used instead of the powdered pigments. Twice as much paste is required as powder, as the paste is made up in resin, and therefore not so concentrated. Although the pastes are easier to mix into the resin than the powdered pigments, they are more affected by the acid used to destroy the heart. Therefore the final cast has a somewhat better appearance if powdered pigments are used.

Before the accelerator is added to the bulk of the two resin mixtures, about 25 ml. of each is poured into small beakers and placed in the refrigerator. This resin, which is needed later, keeps in serviceable condition in the refrigerator for several days. By following this procedure the extra work of preparing some more coloured resin is avoided.

A test (see p. 113) is made to establish the working life of the two resin mixtures. The injection is commenced six minutes before the resin is expected to gel. The resin is run first into the left side of the heart. The openings of the escape holes (via the aorta and the other left pulmonary vein) are held up vertically and, as soon as resin begins to escape from them, they are closed. The same procedure is applied to the right side. Then all escape tubes are held upright for about a minute to allow any water still trapped in the cavities of the heart to float up to their openings. The four screw clamps controlling the openings are then opened slightly one by one, and closed again as soon as resin begins to escape from them. Then the four escape tubes are held vertically until the resin gels, so that any water still in the four cavities of the heart will float up to them.

About half an hour after the resin has gelled, before it has become really hard, the polythene tubes are cut through fairly close to the heart with a strong pair of scissors. The heart is supported in the water by cotton wool to prevent distortion of the casts of the blood vessels which might be caused if it

rested on the bottom of the receptacle. The heart must be handled with the very greatest care at all times after the resin has been injected, to avoid damage to the delicate casts of the blood vessels.

It is necessary to fix the casts of the left and right sides together, before the heart is destroyed in acid, as otherwise the two pieces would fall apart. This work is done the day after the resin was injected. The level of the water in which the heart rests is lowered and the position of the heart arranged so that the ascending aorta, the superior vena cava and the pulmonary trunk are above the water. The heart should not actually be removed from the water, as the latter supports much of its weight. Part of the cast of the ascending aorta, and the adjacent parts of the casts of the superior vena cava and the pulmonary trunk are exposed by dissecting the walls of these vessels away. Small pieces of transparent resin, shaped by filing so that they fit into the gaps between the aorta and the superior vena cava, and the aorta and the pulmonary trunk, are cemented to the coloured cast by means of a little of the coloured resin placed in the refrigerator before the heart was injected.

The heart is left overnight while the cement hardens. Then it is removed from the water and a 3 mm. diameter hole is drilled into the place where it is later intended to fix the mounting rod.

The surface of the heart is gently palpated to discover if any of the casts of the vessels have been broken. If they have, the broken ends are repaired with coloured resin cement, which is applied to the broken ends after the adjacent walls of the vessels have been dissected away. The coronary sinus is opened, as water tends to be trapped here. If the sinus is incompletely filled with resin, the cavity is topped up.

After leaving the heart for eight days for the resin to mature, the tissues are macerated in concentrated hydrochloric acid. During maceration the heart is suspended in the acid, as otherwise the weight of the cast of the cavities might cause the cast of some of the vessels to break, when they are no longer supported by the muscle of the heart. Maceration is completed in about three days if fresh acid is used.

The macerated tissues are washed away, and the cast cleaned and sprayed with a Marco resin 28 C mixture, by the method described on page 123.

Owing to the weight of these casts, it is advisable to mount them in the position in which they hang freely on the mounting rod, rather than to fix them in the anatomical position, unless two rods are used to support them. Figure 45 shows the inferior aspect of a cast of the cavities and blood vessels of a heart, made by this method.

FIG. 45
Photograph of the inferior aspect of a cast of the cavities and blood vessels of the heart. × ⅔.

2. THE CORONARY ARTERIES

A relatively large heart in fresh condition is best for this technique. The ascending aorta is cut through about one inch above the level of the aortic valves. Portex polythene cannulae are tied securely into each of the coronary arteries. After the blood has been washed out of the chambers of

the heart, deaerated water is injected by means of an enema syringe into each of the coronary arteries, to remove all traces of blood from them.

The cavities of the heart are loosely packed with wet cotton wool, introduced in small pieces with forceps via the cut ends of vessels, in order to prevent the wall collapsing during fixation. Then some 5 per cent formalin is run into each coronary artery, and the heart is placed in a tank of 5 per cent formalin for forty-eight hours.

Before the resin injection is commenced, the cotton wool is removed with forceps from the cavities of the heart, and the coronary arteries are again flushed out with deaerated water. Then the heart is placed in water at about 30°C, and left in this long enough for the tissues to be warmed right through.

As perfect a cast as possible is needed to outline the form of the heart, after the latter has been destroyed in acid. To achieve this the inhibition of setting of the resin must be reduced to the minimum, by using a resin mixture with an exceptionally short working life. Although such mixtures heat up considerably before they gel, this does not matter when only small vessels are being filled.

If the room temperature is 20°C, the following mixture is used :

Marco resin 26 C	100 g.
Monomer C	30 g.
Catalyst H C H	6 g.
Red lake pigment powder M. 11	1 g.
Accelerator E	6 ml.

Four units of this mixture are prepared. If the room temperature is over 20°C somewhat less accelerator is added, as the working life of the mixture would otherwise be inconveniently short.

The usual test (see p. 113) is made with one unit of this mixture to determine the exact working life. One unit (*i.e.* 137 g.) is placed in the refrigerator for future use. The remaining two units are used to fill the coronary arteries.

The injection is made by means of the Standard injection apparatus (see Fig. 29, p. 115). A separate apparatus is used to fill each artery, but the two are injected simultaneously.

As a mixture with a very short working life is used, it heats up so much before it gels that the rise in temperature can be used, as well as the information obtained from a test to establish the working life, as an additional guide in deciding the right moment at which to start the injection.

If the room temperature is 20°C, and the working life of the mixture according to the test is ten minutes, the injection is commenced either five minutes after the accelerator is added, or when the temperature of the resin rises to 25°C, whichever is the shorter time. This method of timing the injection compensates for variation between the working life of the test sample and that of the rest of the mixture. A considerable injection pressure is applied alternately to each of the arteries for periods of half a minute until the resin gels. The total amount of resin injected is very small, so that success cannot be judged from this.

About half an hour after the resin has gelled, the Portex cannulae are cut through, about ½ inch being left projecting into the aorta. The heart is left overnight in water, resting on cotton wool.

The next day the ligatures holding the cannulae into the coronary arteries are cut, and the cannulae withdrawn from the resin casts which fill them. The three aortic valves are sewn together, great care being needed to avoid tearing them. Any hole resulting from a tear is plugged with a wad of cotton wool soaked in a rather concentrated gelatine solution. The lips of the valves and the stitches are painted with gelatine solution. When this has set, a little formalin is poured over the gelatine and the specimen left for two hours while the gelatine is hardened by the formalin. This work is done with the heart resting on cotton wool and immersed in water except for the ascending aorta, so that most of its weight is supported.

Next a small amount of the resin which was placed in the refrigerator before the arteries were injected, is transferred to a porcelain palette, and one or two drops of accelerator added and stirred in. The resin mixture is then run into the cusps of each of the valves, so that they are pressed more firmly together. Just before the resin gels, sufficient is added to cover the valves completely. By proceeding in this way the risk is avoided of a considerable quantity of resin flowing into the ventricle while the aorta is being filled. The remaining cavity of the ascending aorta is then filled with resin.

About two hours after this resin has solidified, the coronary arteries are opened at their point of origin from the aorta, and if it is found that the spaces originally occupied by the ends of the cannulae have not been filled with resin added later, this defect is made good with some more of the reserve of resin. The injected heart is then left immersed in water and supported by cotton wool for eight days.

Maceration of the tissues in concentrated hydrochloric acid must be very thorough. The specimen must not be lifted out of the acid while a considerable

amount of macerated tissue is still clinging to the cast of the arteries. The vessel containing the acid is placed in the sink, and cold water is run into it, so that acid and water overflow, and the macerated tissues are washed away. When most of the macerated tissue has been dislodged from the cast, washing is completed in the usual way, except that only a relatively gentle stream of cold water is directed on to it. Some of the macerated tissue may have to be teased away with forceps.

By the method described here a very clean cast, which includes quite tiny branches, can be obtained. The cast is sprayed with a Marco resin 28 C mixture and mounted by the method described in Chapter 17. The resin spray somewhat strengthens the more delicate branches of the cast. The finished specimen must, however, be regarded as rather fragile, and be handled with appropriate care when taken down from the museum shelves. Figure 46 shows a cast of the coronary arteries prepared by this method.

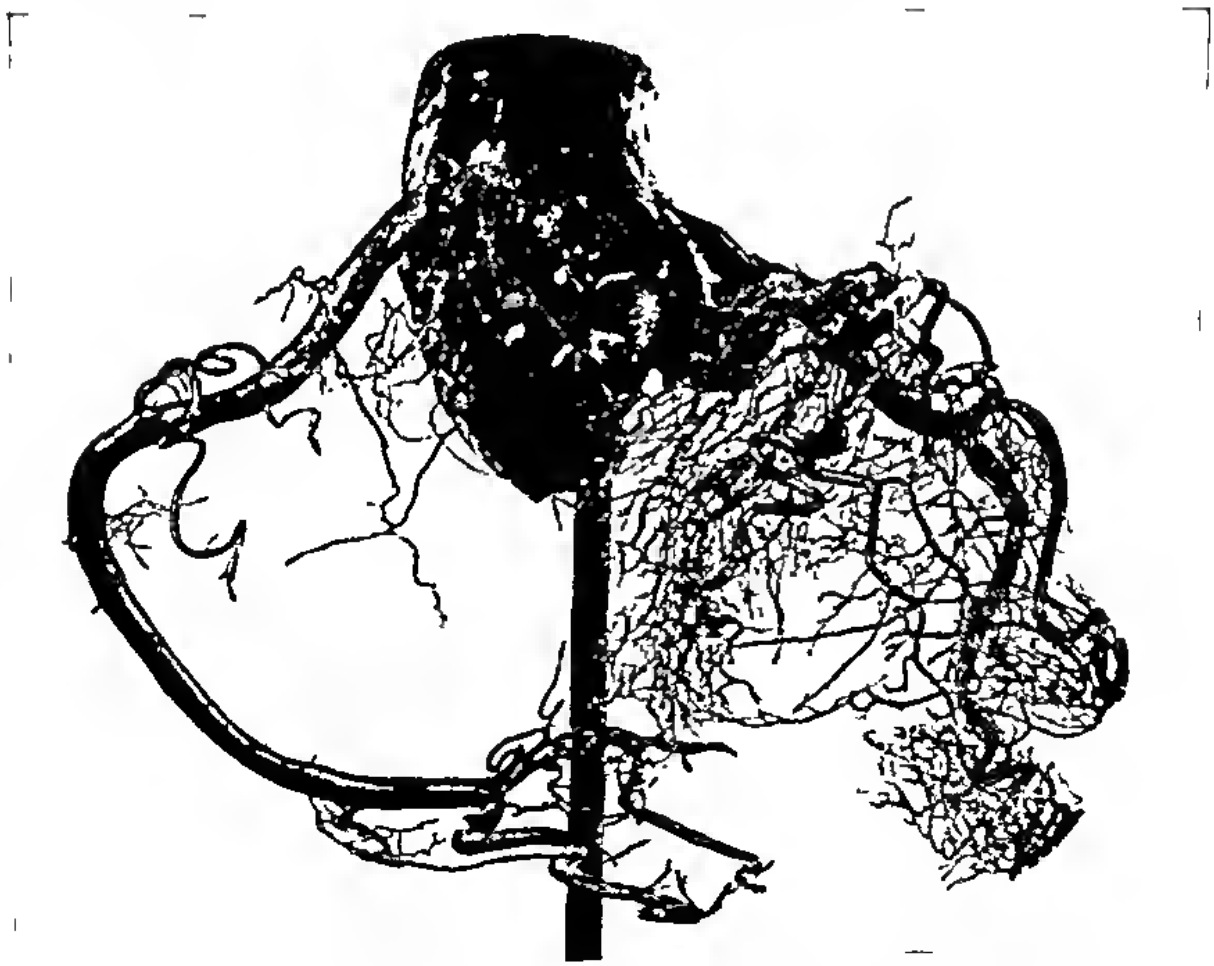

FIG 46

Anterior view of a cast of the coronary arteries × ¾. The finer branches of the right artery were not filled with resin, owing to the presence of blood clots.

Chapter 20

THE PREPARATION OF MODELS IN RESIN

1. INTRODUCTION

FOR more than a century models have been used for teaching anatomy. Unfortunately many of the models produced commercially have been inaccurate. Those with the detailed knowledge necessary for the production of really accurate models usually lack information about the most suitable materials and methods for this work. In this chapter practical details of the simplest methods by which anatomical models can be made are given, for the benefit of those who may wish to try their hand at this fascinating work.

Dental modelling wax is the most suitable material for general purposes, as it is easy to use, and sufficiently rigid to permit quite delicate structures to be modelled with it. Provided they are given a general idea how to handle modelling wax, most people can acquire a considerable amount of skill in this work, which lends itself very much to the development of individual ingenuity.

Wax models are fragile and, if used for demonstrations, they soon get broken. It is desirable therefore to reproduce the original wax models in some stronger material. Mareo resin is particularly suitable for this purpose. The methods recommended are somewhat laborious, but relatively simple to master, even by those who have had no previous training. The construction of a model *of the ossicles of the ear and the tympanic membrane, enlarged 10 diameters,* has been selected to illustrate the general procedure and special problems most frequently encountered in this type of work. Figure 47 shows the completed resin cast of the model.

2. MODELLING IN WAX

No. 4 toughened wax, supplied in sheets packed in 1 lb. boxes, manufactured by the Dental Manufacturing Co. Ltd. (see Appendix), is suitable for all anatomical modelling.

This wax melts at 59°C. At the normal room temperature of 20°C it is quite hard, but when warmed to about 45°C it becomes sufficiently plastic to be modelled by pressure with the fingers.

If a greatly enlarged model is to be made, as in the case of the ossicles of the ear, careful outline drawings are first made to the required scale. The drawings are made with the aid of either a low power binocular microscope or with binocular magnifying spectacles (see Fig. 11, p. 35). The general proportions of the drawings are checked by measurements.

FIG 47

Resin cast, reproduced from wax models, of the ossicles of the ear and the tympanic membrane The models were made ten times linear the size of the actual ossicles

The next step is to make a solid block of modelling wax roughly the same size and shape as the model. In the case of a compact model like the malleus the block can be made by constructing a crude hollow mould with Plasticine, which is built up on a base consisting of a small sheet of glass. Modelling wax is melted in a beaker, and poured into the mould. When the wax is quite cold, the Plasticine is removed. In the case of a relatively flat structure

like the tympanic membrane, the modelling is commenced by cutting an oval-shaped piece out of a wax sheet.

Before modelling is commenced, the wax must be warmed right through to a temperature of about 45°C. This can be done either by immersing the wax in water at about 45°C until the block is warmed, or by holding the block in front of an infra red lamp, and rotating it so that it is warmed equally on all sides. The distance the block is held away from the lamp is adjusted to produce the desired degree of plasticity in the wax. If the wax gets too soft it becomes unmanageable.

While warm, the wax can be shaped by squeezing it with the fingers, and pressing its surface with various instruments. Pieces of wax can be welded on to the main block by holding them in the required position and momentarily melting the two surfaces to be joined with the blade of a scalpel which has been heated in the bunsen. The hot blade is passed between the two surfaces to be joined, while the latter are held in apposition. If the scalpel blade is at the right temperature, it passes easily through the wax, which is melted momentarily, and solidifies almost instantly.

Any cracks which appear in the wax during modelling are repaired by momentarily melting the wax with a heated scalpel blade, or a needle mounted on a handle. Air trapped in the wax is removed by melting the wax with a hot needle, so that the air bubbles come to the surface. It is important to see that the model is composed of a really solid block of wax, rather than a number of pieces held insecurely together in places. In the latter condition the model is fragile, and consequently difficult to work on without breaking it.

The final modelling is best done with the wax quite cold, by scraping or carving away the surplus. The surface of the almost completed wax model can be smoothed by applying a rounded metal surface, such as the handle of a teaspoon, heated to such a temperature in the bunsen that when lightly drawn across the surface of the wax it momentarily melts it. Finally the surface is polished with a wad of cotton wool soaked in benzene which dissolves the surface of the wax.

The incus is modelled in the same way as the malleus. In order to get an articular surface on the incus which fits that of the malleus, and at the same time to ensure that the models of the two bones are correctly related to each other when the articular surfaces are brought together, the model of the incus is warmed until plastic, and then its articular surface is firmly pressed against that of the model of the malleus, the latter being cold. In this way the articular surface of the model of the incus is pressed into the shape required.

While the two models are held in apposition, the short and long processes of the incus can also be bent into their correct position relative to the malleus. (In order to determine the correct positions, it is necessary to articulate the bones themselves, which fit together perfectly.)

No special problems are encountered in modelling the stapes except that, being rather a delicate structure, it is easily broken. It is not however practicable in this case to start with a block of wax. Strips cut from a sheet of wax are softened by holding them either over a bunsen flame or in front of an infra red lamp until they are plastic. Then they are pressed into the rough shape, and welded together before more wax is added.

The thickened rim round the tympanic membrane is built up by welding strips of wax on to the margin of the oval sheet from which the model of the membrane is made. If it is desired to represent the membrane as smoothly conical, this is most easily done by preparing a suitable block of Dentruset plaster and shaping it by scraping it with a scalpel and smoothing it with abrasive paper. The model of the tympanic membrane is then shaped by pressing the warm wax sheet on the plaster former.

3. PREPARATION OF NEGATIVE MOULDS

Although Dentruset plaster can be used to make negative moulds, Kaffir D plaster (see Appendix) being harder, is more satisfactory. Unless only one resin cast is to be made, the negative moulds must be so constructed that there are no undercuts, the presence of which makes it impossible to remove the cast without breaking the mould. Each mould consists of a minimum of two pieces, but frequently it is necessary to make them in three or more pieces, in order to avoid undercuts. Careful thought is required to decide how many pieces are necessary, and the extent of each piece. Two initial attempts at this work may be necessary before a successful result is obtained.

The design of the mould must be such that any long slender process on the model lies longitudinally half in one piece and half in another. Otherwise, even though there are no undercuts, the process is very likely to break off when the wax model or the cast is removed from the mould. This point is well illustrated in the construction of the negative mould for the model of the malleus, which has three slender processes (see Fig. 49).

The moulds are made in the following way. The wax model is partly embedded in Plasticine, which is first pressed on to a rectangular piece of glass. As much of the model is allowed to project above the Plasticine as is possible

without causing undercuts to be produced when the projecting part is embedded in plaster. The Plasticine is pressed and smoothed around the model so that it is surrounded by a more or less horizontal rim of Plasticine. Oblong slots are cut in the Plasticine rim, which produce corresponding projections in the piece of plaster. These keys lock into place the other piece or pieces of plaster of which the completed mould is made. The wall of the Plasticine rim is made perpendicular to the glass base, by scraping it with a glass slide. If the Plasticine sticks to the instruments used to shape and smooth it, this is prevented by the application of a little oil to the surface.

FIG. 48

Wax model of the malleus, partly embedded in Plasticine, just before the cardboard wall is fixed around it. The cardboard forms the wall of a chamber above the model, into which the plaster is poured, in the first stage of the construction of the negative mould.

Next the Plasticine is surrounded by a wall made of a strip of very thin cardboard, impregnated with paraffin wax to make it waterproof. The wall must project about an inch above the highest part of the model and is held in place by Sellotape, reinforced by Plasticine applied to the outside, by pressing it against both the glass base and the cardboard. Figure 48 shows the model of the malleus, partly embedded in Plasticine, just before the cardboard wall is fixed in position. The cardboard forms the wall of a chamber which is filled with plaster, to form the base-piece of the negative mould.

Before the plaster is poured into the chamber, the whole of the inside of the latter is painted with water to which a sufficient number of drops of 20 per cent Manoxol O.T. (see Appendix) have been added, to ensure that the water wets the whole surface, instead of pulling away into droplets. The

application of this wetting agent prevents air bubbles clinging to the surface of the model and the Plasticine when the plaster is poured in.

Kaffir D plaster is prepared for use by mixing 100 parts by weight of the plaster with 40 parts of cold water. The water is placed in a rubber mixing bowl and the plaster added. The mixture is stirred with a plaster mixer until the mixture has a creamy consistency without lumps. (It is important that the plaster be added all at once, as this procedure produces a harder and tougher plaster cast than if the powdered plaster is added gradually.) Next, the bottom of the bowl is sharply struck a number of times, in order to bring to the surface as much as possible of the air in the mixture. Then sufficient plaster is poured into the chamber constructed of plasticine and cardboard, to immerse the highest part of the model to a depth of about half an inch. The glass base of the chamber is now tapped and shaken until the plaster sets, to get rid of any air bubbles still in the mixture.

The plaster sets about fifteen minutes after mixing. After about thirty minutes it will be sufficiently hard for the cardboard to be removed, and for the piece of plaster to be lifted away from the model and the Plasticine. If too much of the wax model has been allowed to project above the Plasticine, so that undercuts are produced, the wax model comes away adhering to the plaster. If this happens the edges of the plaster next to the wax are scraped away, before the plaster gets really hard, until the undercuts are eliminated and the wax model can be detached.

The piece of plaster is placed overnight in a drying oven at a temperature of about 45°C. When it is quite dry, the cavity and flange around it are given two coats of a 20 per cent solution of orange flake shellac dissolved in industrial spirit, applied at intervals of about an hour. Sufficient shellac is applied to produce a shiny surface.

The piece of plaster is left overnight for the shellac to become quite hard. Then the shellacked surface is coated with a thin film of vaseline, which acts as a separating medium. Next the piece of plaster is placed on the table with the shellacked surface uppermost, and the wax model is replaced in it.

The plaster is surrounded with a strip of waxed cardboard. If it is necessary to complete the mould in more than one piece, a Plasticine wall is built between the opposite sides of the waxed cardboard and carefully pressed against the plaster and wax model, so that a chamber is formed into which the plaster forming the next piece of the mould can be poured.

The second piece of plaster is made in the same way as the first. The third piece of plaster, which completes the negative mould, is made by

without causing undercuts to be produced when the projecting part is embedded in plaster. The Plasticine is pressed and smoothed around the model so that it is surrounded by a more or less horizontal rim of Plasticine. Oblong slots are cut in the Plasticine rim, which produce corresponding projections in the piece of plaster. These keys lock into place the other piece or pieces of plaster of which the completed mould is made. The wall of the Plasticine rim is made perpendicular to the glass base, by scraping it with a glass slide. If the Plasticine sticks to the instruments used to shape and smooth it, this is prevented by the application of a little oil to the surface.

FIG. 48

Wax model of the malleus, partly embedded in Plasticine, just before the cardboard wall is fixed around it. The cardboard forms the wall of a chamber above the model, into which the plaster is poured, in the first stage of the construction of the negative mould

Next the Plasticine is surrounded by a wall made of a strip of very thin cardboard, impregnated with paraffin wax to make it waterproof. The wall must project about an inch above the highest part of the model and is held in place by Sellotape, reinforced by Plasticine applied to the outside, by pressing it against both the glass base and the cardboard. Figure 48 shows the model of the malleus, partly embedded in Plasticine, just before the cardboard wall is fixed in position. The cardboard forms the wall of a chamber which is filled with plaster, to form the base-piece of the negative mould.

Before the plaster is poured into the chamber, the whole of the inside of the latter is painted with water to which a sufficient number of drops of 20 per cent Manoxol O.T. (see Appendix) have been added, to ensure that the water wets the whole surface, instead of pulling away into droplets. The

FIG. 50

Wax model of the tympanic membrane, and the two pieces of plaster, of which the negative mould is composed

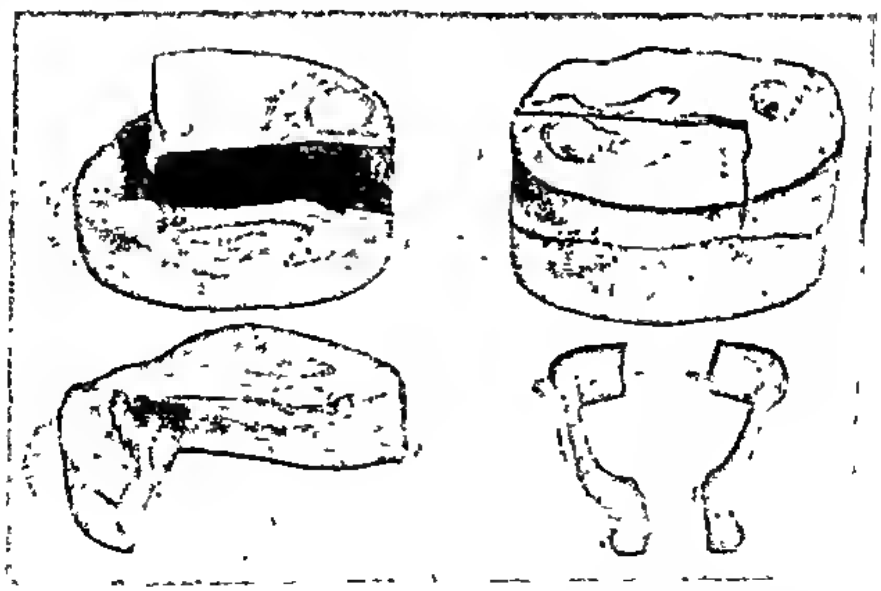

FIG 51

Wax model of the stapes, which has been cut into two pieces to facilitate the construction of plaster negatives The negative moulds of the two pieces are shown

following the same procedure. Finally a filling hole and any escape holes which may be necessary to allow air to escape when the mould is being filled with resin, are drilled in the two pieces which form the top of the mould. The holes are drilled from the inside, as when the drill emerges from plaster, it

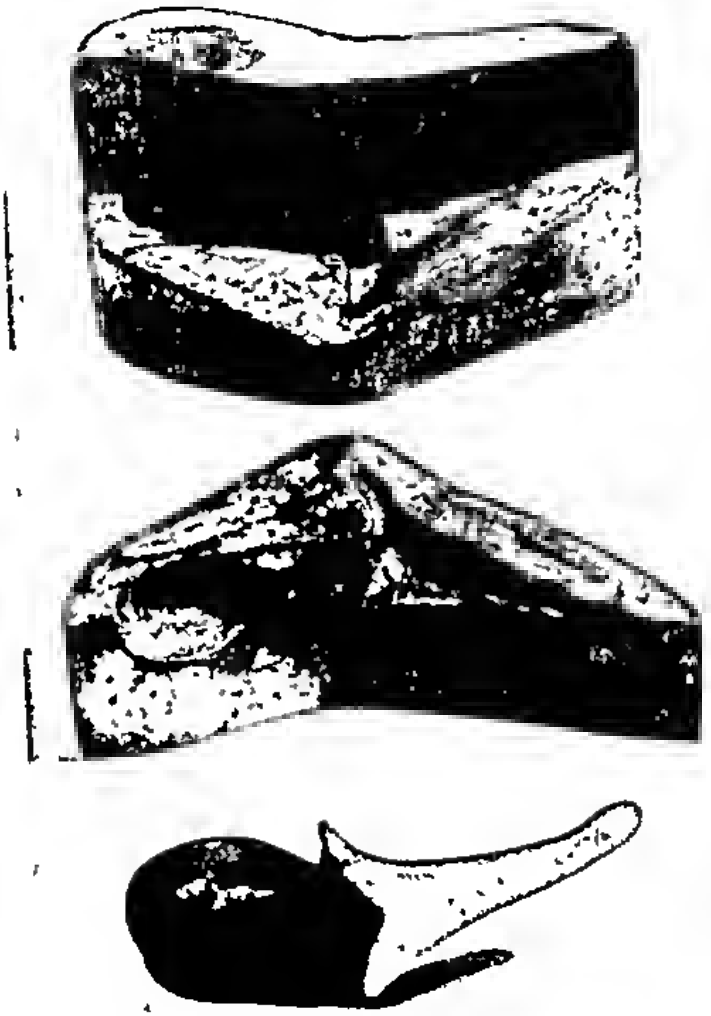

FIG 49

Wax model of the malleus, and the three pieces of plaster, of which the negative mould is composed

usually breaks a small fragment of plaster away. The outside of the filling hole is enlarged with a counter-sinking drill so that it is cone-shaped. Figure 49 shows the model of the malleus, and the three parts of the plaster mould.

The negative mould of the model of the incus is constructed in exactly the same way as that of the malleus. The mould of the model of the tympanic membrane can be made of two pieces, as shown in Figure 50. Note the filling hole and air escape hole in the upper part of this mould.

The solution is applied cold with a sable water-colour brush to all shellacked surfaces of each mould, including the filling and escape holes. The separate parts of the mould are left for two days for the polyvinyl alcohol to harden before the mould is assembled.

After the two or three parts of a mould have been assembled, a length of Sellotape is wrapped right round the vertical wall of the mould, over the junction between the upper and lower halves. Next the mould is placed on a sheet of glass, and a sheet of Plasticine, previously rolled out flat on a sheet of plate glass in the same way that pastry is rolled out, is wrapped round the vertical walls. The Plasticine is pressed firmly against the glass base and against the walls of the mould. It must extend just above the upper surface of the mould. The purpose of the Sellotape and the Plasticine is to hold the pieces of the mould firmly together, and to prevent leakage of resin. The glass base facilitates handling of the mould, without risk of disturbing the Plasticine.

The following resin mixture is suitable for filling the moulds :

Marco resin 26 C	100 g.
Monomer C	35 g.
Catalyst H C H	4 g.
Crystic resin 182 (plasticiser)	10 g.
Accelerator E	1 ml.

Pigment paste or powder is added as desired. White with a trace of yellow gives a satisfactory colour for casts of the ossicles, but transparent resin is more suitable for the cast of the tympanic membrane. The inclusion of the plasticiser is not essential, but it makes the casts less brittle.

Before the accelerator is added to the coloured and clear resin, to be used to fill the moulds, a little of each is decanted into small beakers, and placed in the refrigerator. This is used later, for filling up any air holes in the casts, and for cementing the two halves of the stapes together.

At a room temperature of 20°C the mixture has a working life of about 2 hours, unless pigments are used which materially affect the working life. In the latter case some adjustment of the amount of accelerator added may be necessary.

After the accelerator has been stirred in, the mixture is left standing for fifteen minutes until all air bubbles have risen to the surface. The resin is run into the moulds from apparatus consisting of a funnel, to which a slender Portex polythene delivery tube is connected by rubber tubing. The rate of

Owing to difficulties concerning undercuts, it is necessary to cut the model of the stapes into more or less symmetrical halves, and make a separate mould of each half, as shown in Figure 51. The resin casts of the two pieces of the stapes can be cemented together so that the join is almost invisible.

Finally the separate parts of each mould are assembled, and the walls rubbed with abrasive paper so that, when the mould rests on the table, they are smooth and vertical. Traces of vaseline are removed from the shellacked surfaces by wiping them with a wad of cotton wool soaked in benzene.

4. PRODUCTION OF RESIN CASTS FROM NEGATIVE MOULDS

The cavity and the surfaces of the mould which come in contact with each other, are carefully examined, to see that they have a continuous shiny skin of shellac; if not, another coat of shellac is applied, and the parts left for twenty-four hours for the shellac to harden. The shellac serves two important purposes. It closes the pores in the surface of the plaster, facilitating the application of the next coat, and also enables the parts of the mould to be separated after they have been filled with resin and the latter has hardened. The separation is achieved by soaking the moulds in spirit, which dissolves the shellac.

It is necessary to cover the shellac with a film of a suitable type of polyvinyl alcohol, such as Mowiol 50-88 (see Appendix), as resin clings to shellac and also renders it quite insoluble in spirit. The following solution is used for this protective film :

Mowiol 50-88	15 g.
Water	100 ml.
Glycerine	0.5 g.
Manoxol O.T. 20% solution	2 ml.
Formalin	2 ml.

The glycerine is added as a plasticiser, to prevent the film of polyvinyl alcohol from cracking. The Manoxol is a wetting agent, which enables an even film of the solution to be applied. The formalin is a preservative. This solution keeps indefinitely.

Mowiol 50-88 is not readily soluble in water. It is dissolved by heating the water to a maximum temperature of not more than 80°C and by stirring continuously to avoid local overheating.

The solution is applied cold with a sable water-colour brush to all shellacked surfaces of each mould, including the filling and escape holes. The separate parts of the mould are left for two days for the polyvinyl alcohol to harden before the mould is assembled.

After the two or three parts of a mould have been assembled, a length of Sellotape is wrapped right round the vertical wall of the mould, over the junction between the upper and lower halves. Next the mould is placed on a sheet of glass, and a sheet of Plasticine, previously rolled out flat on a sheet of plate glass in the same way that pastry is rolled out, is wrapped round the vertical walls. The Plasticine is pressed firmly against the glass base and against the walls of the mould. It must extend just above the upper surface of the mould. The purpose of the Sellotape and the Plasticine is to hold the pieces of the mould firmly together, and to prevent leakage of resin. The glass base facilitates handling of the mould, without risk of disturbing the Plasticine.

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At a room temperature of 20°C the mixture has a working life of about 2 hours, unless pigments are used which materially affect the working life. In the latter case some adjustment of the amount of accelerator added may be necessary.

After the accelerator has been stirred in, the mixture is left standing for fifteen minutes until all air bubbles have risen to the surface. The resin is run into the moulds from apparatus consisting of a funnel, to which a slender Portex polythene delivery tube is connected by rubber tubing. The rate of

flow of resin is regulated by means of an adjustable screw clamp attached to the rubber tubing. The apparatus is shown in Figure 52.

The resin must be run in at a steady flow, regulated so that the cone-shaped filling hole is kept full all the time. If the flow is intermittent, or too slow, air and resin will be drawn into the mould together. While each mould is being filled, it is tilted in such a way as to facilitate the complete displacement of the air by resin.

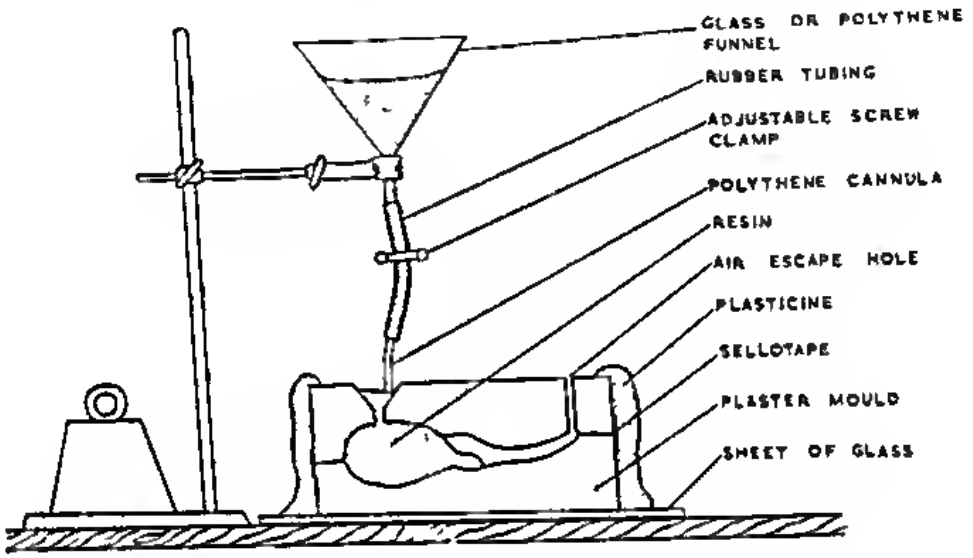


FIG 52

Diagram to show the method of filling a plaster negative mould with resin. The mould is shown in sectional view.

When the mould is completely full, loosely fitting pieces of wire are placed in each of the holes. The wires displace most of the resin in the holes, and so facilitate the subsequent detachment of the resin cast from the plaster. But they must be removed soon after the resin gels as, if left in position until it is really hard, they are cemented so firmly into the holes that it is impossible to withdraw them without damaging the plaster.

The moulds are left for four days while the resin hardens. Then the Plasticine and Sellotape are removed and the moulds are immersed in industrial spirit for twenty-four hours. Then they are removed from the spirit and the individual parts gently pulled apart. If they do not readily separate, they are returned to the spirit for a further twenty-four hours as, if force is used to separate them, the moulds may be damaged.

After the resin casts have been removed, the pieces of plaster are gently brushed with spirit, until all traces of shellac have been removed. They are dried in an oven at about 45°C, before being prepared for future use.

If a piece is broken off a plaster mould, it can be stuck on again with a cement based on Marco resin 28 C (see p. 196). This resin is more suitable for this particular work than Marco resin 26 C.

The resin casts are washed in hot water to remove any polyvinyl alcohol adhering to them. Excess resin at the junction between the individual parts of the mould is filed away and any air holes are filled with some of the resin previously placed in the refrigerator for this purpose.

Finally, any defects on the surface of each cast are removed by rubbing with various grades of waterproof carborundum paper, finishing with grade 500, which produces a smooth matt surface.

The separate casts are cemented together with resin cement. While the cement is setting the individual casts are held in their correct positions with Plasticine.

Chapter 21

CASTS FROM THE BRAIN

1. INTRODUCTION

THE first accurate figure illustrating the form of the cerebral ventricles was published by Gustav Retzius in 1900. This figure, which is a composite one, was drawn from a number of incomplete casts of the ventricles, as Retzius found it impossible, using Wood's metal, which was the most suitable injection mass available for this type of work at the time, to obtain a complete cast of what he regarded as typical cerebral ventricles. For the next fifty years Retzius's illustration was reproduced in most standard text books.

The technical difficulties involved in making casts of the cerebral ventricles depend largely on whether the latter are plump, as in Figure 53, or slender as in Figure 54. It is much more difficult to obtain a perfect cast of slender ventricles than plump ones.

When Marco resin is used to make casts of the cerebral ventricles, a complete cast can be obtained every time, no matter what type of ventricular system is being filled. For although the initial injection rarely fills all the cavities, they can be topped up with resin where necessary, during the subsequent removal of the brain from around the cast. The brain is removed by very carefully slicing it away. By this procedure the worker can be certain that the finished cast really represents the full extend of the original cavities.

The first prerequisite for success in making casts of the cerebral ventricles is to fix the brain with the minimum distortion and shrinkage, before the mass is injected.

Several special problems are encountered in the injection of the resin. The cavities to be filled are culs-de-sac. It is impracticable to provide sufficient escape holes to allow all the fluid or air in the cavities to escape freely when it is being replaced by resin. It is equally impossible to avoid certain leakages as, for example, from the third ventricle, resulting from accidental rupture of its roof and floor. In the case of the fourth ventricle, resin often escapes from the foramen of Magendie more rapidly than it enters through the aqueduct, unless the latter is unusually wide. The slender form of some of the cavities

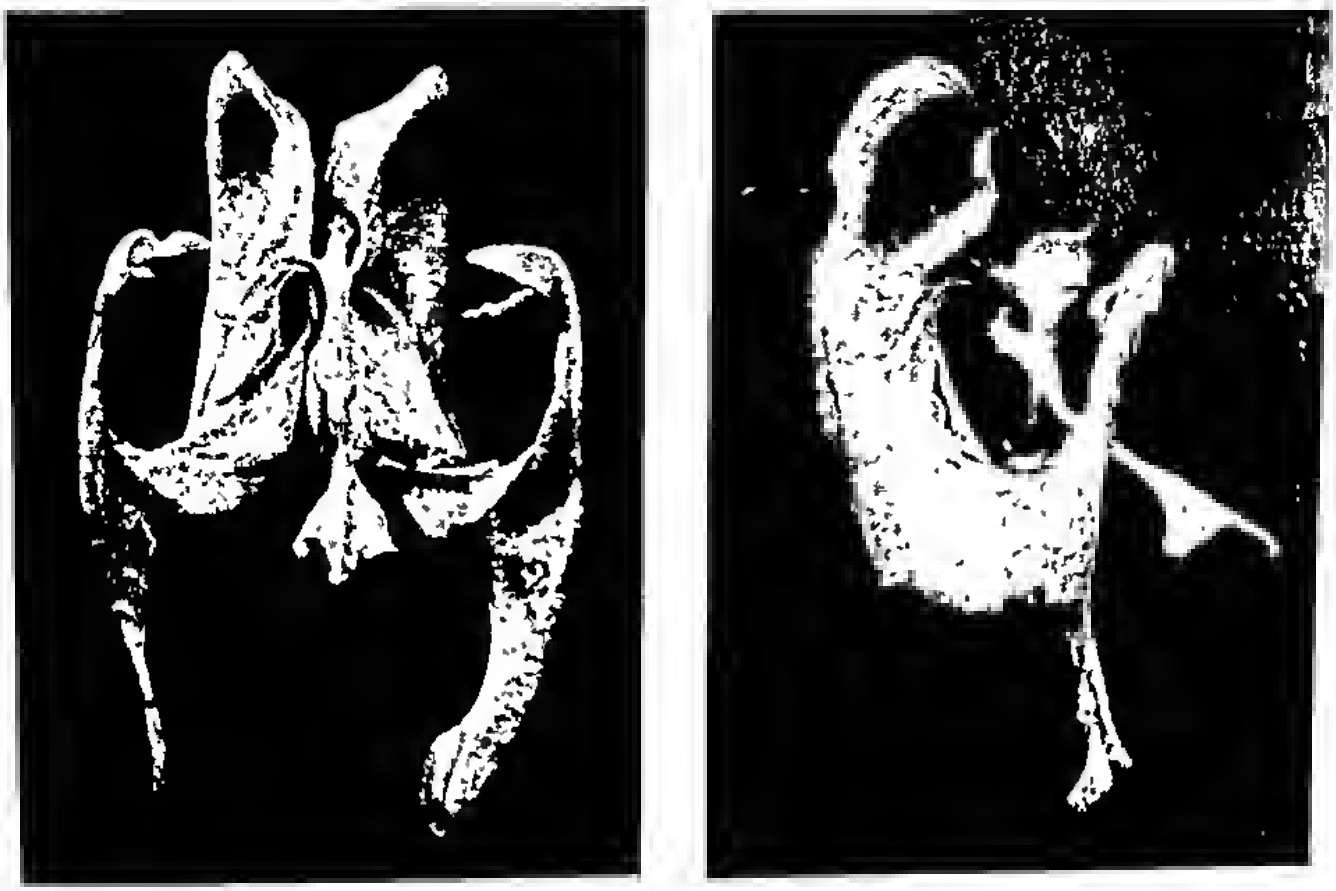


FIG 53

Ventral and right lateral views of a cast of plump cerebral ventricles. × ⅔

FIG. 54

Ventral and right lateral views of a cast of slender cerebral ventricles. × ⅔

greatly increases the difficulty of filling them completely. And even when a perfect cast has been produced, care is needed to isolate it from the brain without damage.

A model of the cerebral ventricles can be made in modelling wax, though this is a somewhat difficult task for those who have had no previous experience in this type of work. As a wax model would soon be broken if used for demonstrations, it is desirable to reproduce it in a stronger material. Marco resin 26 C is suitable for this work.

Once a satisfactory wax model of the ventricles has been made, the production of a hollow model, embedded in a rectangular block of transparent resin, is comparatively easy, though rather laborious.

2. CASTS OF THE CEREBRAL VENTRICLES

(*a*) ADULT

The brains used for this work are removed at post mortem and fixed in 10 per cent formalin, by the method described on page 64. They should be left in formalin for a minimum period of six weeks before the ventricles are filled with resin, but satisfactory results can be obtained after much longer storage in formalin. Although about 1 per cent general shrinkage and 1 per cent distortion occur during fixation, this is insignificant when compared with the natural variation in the size of the ventricles.

When fixed brains are injected with resin, it is impossible to produce an artificial distortion of the ventricles, resulting from excessive injection pressure. For unless extremely low injection pressure is employed, the floor and roof of the third ventricle are ruptured, and resin also escapes from the lateral ventricles as a result of the white matter splitting in the planes through which the fibres run.

Before a fixed and hardened brain is prepared for the injection of the resin, it is useful to take an antero-posterior ventriculogram. This indicates whether the ventricles are plump, and therefore comparatively easy to fill with resin, or slender, in which case special care is needed. Figure 55 A shows a ventriculogram, (taken at a slightly later stage when the injection holes have been made and the injection cannula inserted). This figure shows clearly the type of ventricles. Figure 55 B shows a photograph of the actual resin cast of one of the ventricles made from the same brain, taken before the brain tissue had been removed from around the other ventricle.

The first step in preparing the fixed brain for injection is to drill holes

A B

FIG 55

A. Anteroposterior ventriculogram of a brain with the metal injection tube in position Note the shadow of the escape hole drilled in the opposite hemisphere

B Photograph of the same brain after the injection of the resin, with one half of the brain removed from the cast. × 4 9 (Fig 60 is a lateral view of the same specimen)

into each cerebral hemisphere. The holes are drilled by means of a short length of brass tube, of about 5 mm. internal diameter, one end of which has been sharpened on a hone, so that it resembles a cork borer. The unsharpened end of the brass tube is attached by rubber tubing to the vacuum pump, and the tube is then inserted vertically through the precentral gyrus of each cerebral hemisphere, about 1.5 cm. away from the longitudinal fissure (see Fig. 55 A and Fig. 56). The tube is rotated as it is pressed into the brain to ensure that it cuts cleanly. Except in the case of very slender ventricles, when the tube enters the ventricle, the piece of brain tissue within the tube is sucked into the rubber tubing, and a gurgling noise is heard as the vacuum pump sucks a mixture of air and water out of the ventricle. Before the second hole is cut, the brain tissue must be removed from the rubber tubing, in case it impairs the efficiency of the vacuum pump.

In the case of slender ventricles, the holes are cut to a depth of about 5 cm. If no characteristic noise indicates that the holes extend into the ventricles, the brain is immersed in water and air is gently blown into each hole. If the air blown into one ventricle does not escape via the hole in the other, the depth of each hole is slightly extended. When this is done, special care is needed to prevent fragments of brain tissue, cut off when the tube is

reinserted into one of the holes, from dropping into the ventricle. Such fragments may become lodged in one of the interventricular foramina and jeopardise the success of the resin injection.

Air must always be blown into both holes while the brain is immersed in water. Sometimes when air is blown into one it escapes freely from the other, but will not pass in the reverse direction. This is usually due to a little

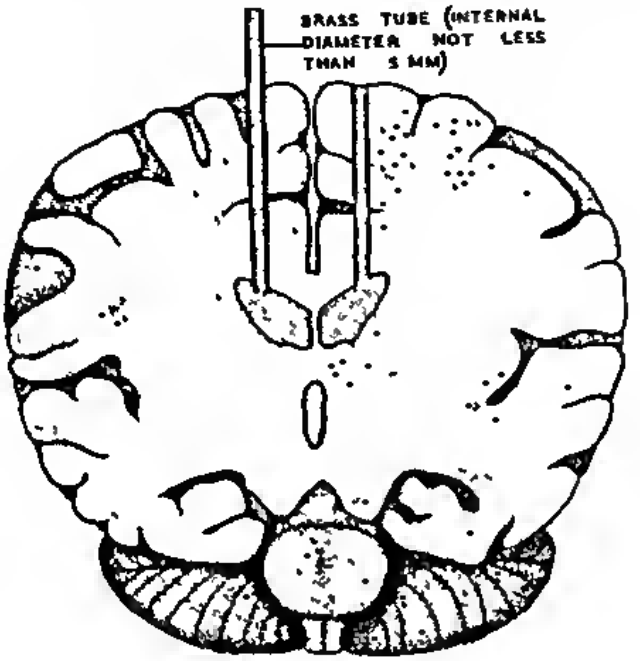


FIG 56

Anterior view of a coronal section of the brain just anterior to the pons, to show the brass tube inserted into one of the lateral ventricles, and a hole bored into the other, in preparation for filling the ventricles with resin.

flap of white matter on the roof of one of the lateral ventricles, which was not cut cleanly away by the boring tube, and which acts as a valve. Such an obstruction can be dislodged by means of a slender probe with a small semicircular blade projecting at right angles from the end.

It is frequently found that the lamina terminalis is ruptured, and the pituitary recess opened through tearing the pituitary stalk during the removal of the brain from the cranium. In order to prevent excessive escape of resin from these places, and from the foramen of Magendie, when the injection is being made, small wads of cotton wool are first placed over these openings, and then the whole base of the brain is coated with Dentruset plaster. The cotton wool prevents the plaster entering the ventricles. The plaster also holds the cerebellum firmly to the cerebral hemispheres.

300 ml. of the following resin mixture are used for the injection of each brain :

Marco resin 26 C	100 g
Monomer C	15 g.
Catalyst H C H	4 g.
Pigment paste	1 g.
or Pigment powder	0.5 g.
Accelerator E	3 ml.

The above formula is suitable for working at a room temperature of 20°C. As a viscous mixture with a working life of about twenty minutes is required, the monomer C and accelerator E content require adjustment if the room temperature is considerably more than 20°C. This is one of the few resin techniques which can be undertaken successfully in comparatively hot weather.

Before the accelerator is added, about 50 ml. of the mixture are decanted and placed in the refrigerator for future use.

The injection is made by gravity flow, using the apparatus shown in Figure 57. The brain is immersed in water at 30°C during the injection. The water supports most of the weight of the brain and so prevents distortion and, as the water is 10°C warmer than the working temperature, resin escaping from leaks gels before the resin in the injection apparatus, and so seals the leaks, while resin can still flow in.

The injection of the resin is commenced as soon as the accelerator has been incorporated and the apparatus filled. The resin is injected by means of the tube used to drill the holes into the lateral ventricles. The tube is inserted into one of the holes and resin is allowed to flow in until it escapes via the other. Then the tube is inserted into the other hole, so that the direction of the flow of resin is reversed. The resin is allowed to flow continuously but not so rapidly that all may be used up before it gels. The rate of flow is adjusted by means of the screw clamp attached to the rubber tubing connecting the funnel to the injection tube (see Fig. 57). The brain is held in various positions and the temporal and occipital lobes are compressed from time to time, in order to displace water and air from the temporal and posterior horns, so that they may be filled with resin. When no more air and water escape from the escape hole in one hemisphere, the direction of flow is again reversed, and the same procedure repeated. It is advisable to wear a pair of household rubber gloves during these manipulations, to protect the hands from the resin. The gloves are cleaned before they are taken off, by wiping away

the resin on them with large wads of cotton wool soaked in Homacol or other liquid detergent.

Resin is allowed to flow into the ventricles via one hole and escape freely via the other until it gels. The escape hole must not be obstructed as, although this is an effective way of forcing resin into the fourth ventricle, it increases the injection pressure sufficiently to rupture the roof of the third ventricle.

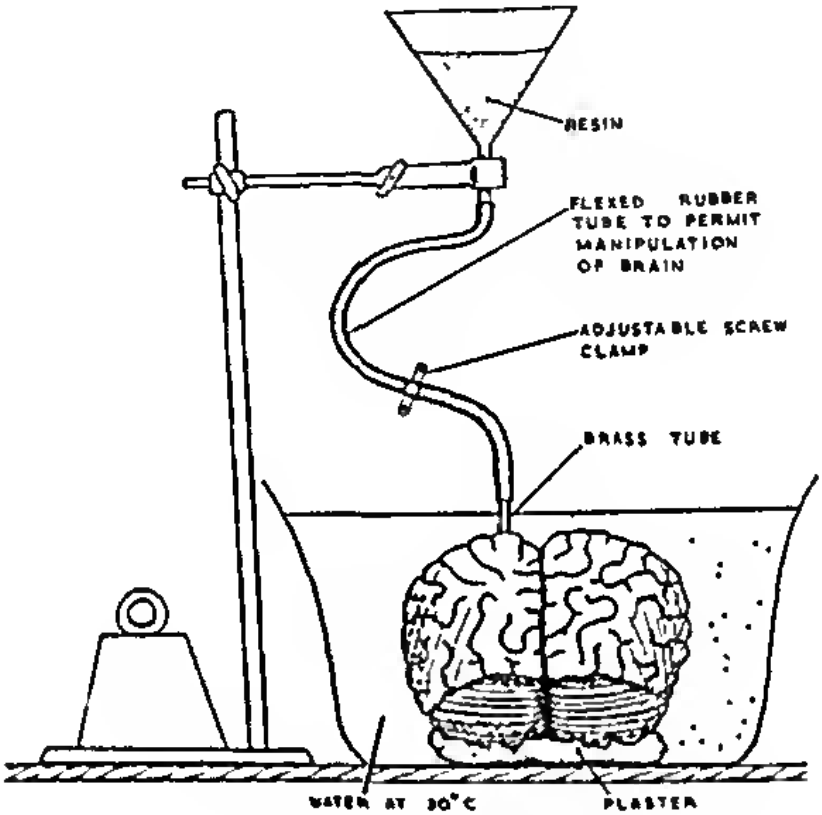


FIG. 57

Apparatus for filling the cerebral ventricles with resin

When the resin has gelled, the injection tube is withdrawn, and the brain is left immersed in water overnight while the resin hardens. After the bulk of the plaster has been removed with bone forceps, the brain is sliced away with horizontal slices from the top, until the level of the bodies of the lateral ventricles is reached. If these are not completely filled, they are topped up with a little of the resin from the refrigerator, after the addition of accelerator. The resin can be run into the cavity from the blade of a scalpel.

As soon as the mixture used for topping up has hardened sufficiently, the work of removing the brain tissue is resumed. This must be done with special care in those areas where experience indicates that topping up is frequently

necessary. The temporal horns are often incompletely filled. If it is desired to preserve in the cast an indication of the full extent of the ependyma, the upper and lower surfaces of which are usually in contact in much of the temporal horns, this can be done by removing the roof of the temporal horn, and applying a thin layer of resin over the area where no cast has been formed. As this area is dome-shaped, consisting of the upper surface of the hippocampus, it is necessary to apply the resin when it is on the point of gelling, and to scrape it back as fast as it flows off the summit of the dome, until the resin gels. It is sometimes necessary to apply the resin in two stages, in order to get an adequate layer. Surplus resin is trimmed away with a sharp scalpel, before it gets too hard to cut. The posterior horns are often incompletely filled, especially if they are very slender. Frequently however posterior horns are absent on one or both sides.

The only part of the third ventricle which is often incompletely filled is the suprapineal recess. This was overlooked in the preparation of the cast shown in Figure 53. The concave surface seen in the lateral view of the cast of the third ventricle of this specimen indicates where air or water prevented the primary injection from filling the recess.

The fourth ventricle is best approached from the side of the brain, by making vertical slices through the cerebellum. More often than not it is incompletely filled. If the aqueduct itself is not filled, its cavity is slightly enlarged, so that, with the brain resting on its side, a little trough consisting of brain tissue is formed. This is filled with resin. Surplus resin is removed from the isolated cast by filing, to reduce the cast of the aqueduct to normal dimensions. The provision of an artificial aqueduct is rather difficult, but fortunately it is very unusual for the aqueduct not to be filled by the primary injection.

Before the completed cast can be freed from the remains of the base of the brain, it may be necessary to remove a certain amount of resin which has escaped from the third ventricle. This is done with bone forceps, after the cast has been soaked in water at about 40°C to make the resin flexible, and so reduce the risk of breaking part of the cast. When this work is done, boldness tempered by judgment is needed. In the event of the cast itself being broken, it can be repaired by means of resin cement.

It is advisable to join the lateral ventricles with a bridge of transparent resin, as this greatly strengthens the cast. A small piece of clear resin is filed until it fits the gap between the lateral ventricles, and is then cemented into place.

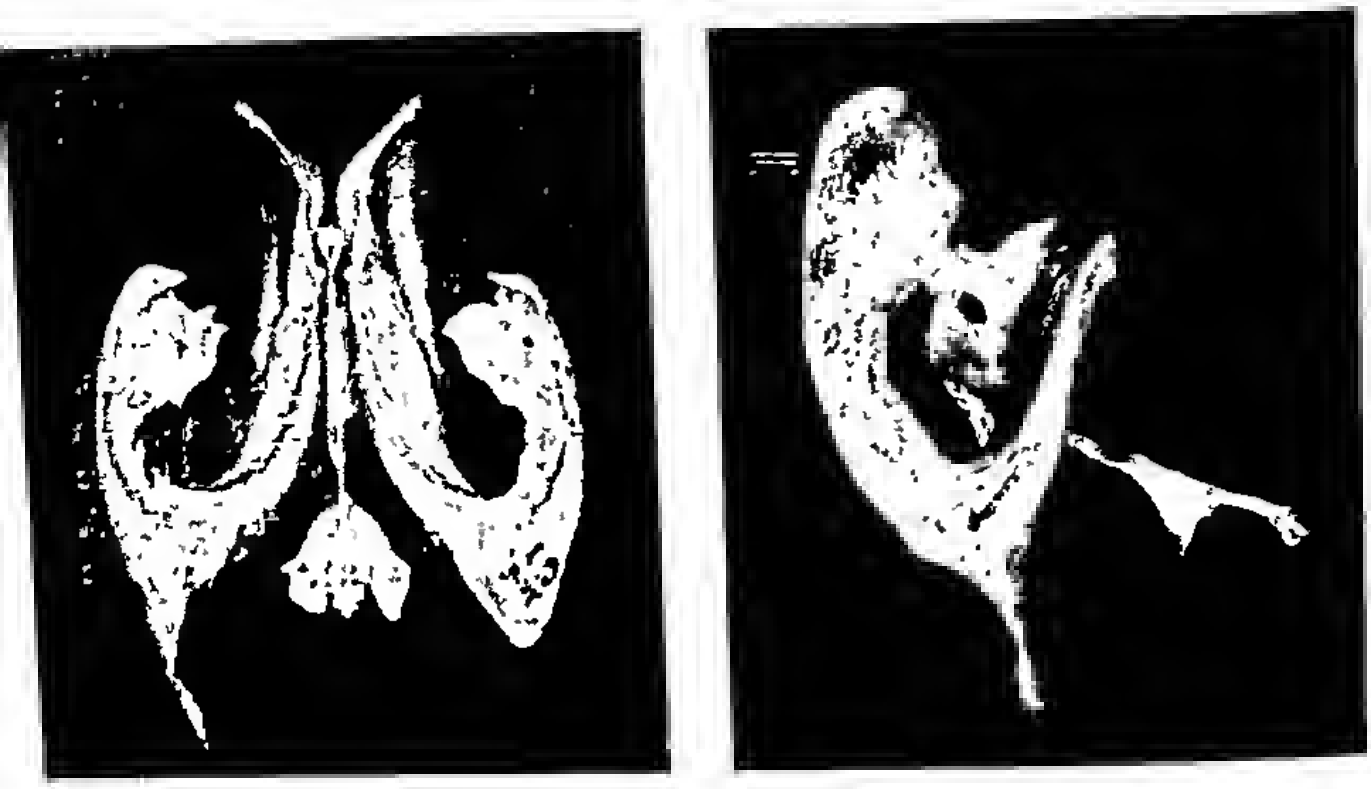
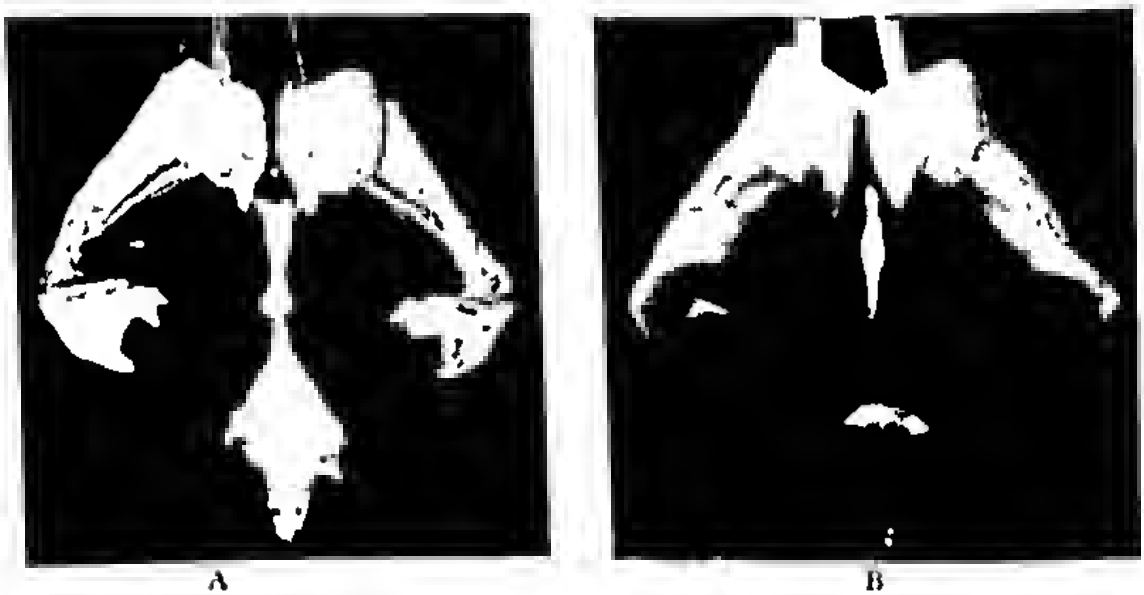
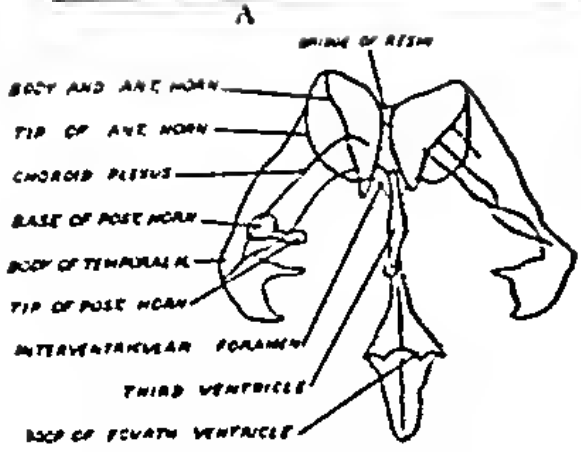


FIG. 58

Ventral and right lateral views of a cast of the cerebral ventricles, selected from among 30 specimens as representing, as far as any one cast can, the typical form of the ventricles. × 3/5.



A B



C

FIG. 59

A. Anteroposterior photograph of a cast (also illustrated in Fig. 58) for comparison with a radiograph. × 4/9

B Anteroposterior projection of the cast.

C. Explanatory diagram of the anteroposterior projection

Figure 58 has been selected from thirty casts to represent typical cerebral ventricles. The choice was based on three considerations: the cast is not too plump, it is not too slender, and the posterior horns are asymmetrical.

The value of the casts as aids in the interpretation of ventriculograms is enhanced if X-ray photographs of each cast are displayed alongside. Without the addition of any radio-opaque material, Marco resin gives an X-ray picture of good contrast for the interpretation of shadows, as shown in Figure 59, which shows an anteroposterior photograph and an anteroposterior X-ray projection of one of the casts.

In order to demonstrate the position occupied by the ventricles in the brain, a wet specimen can be prepared, in which the brain is removed from only one side, as shown in Figure 60.

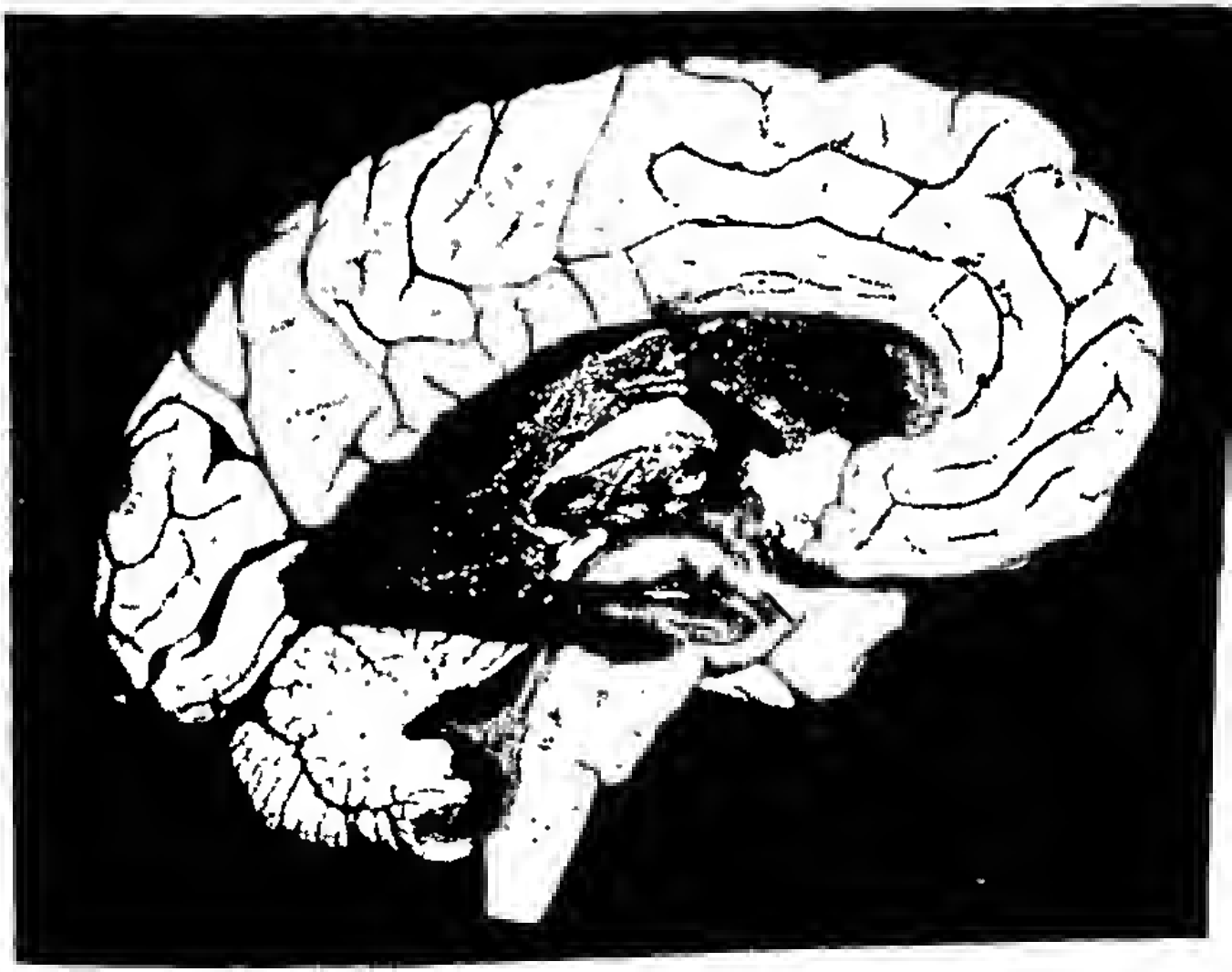


FIG. 60

Lateral view of a cast of the ventricles from which only half the brain has been removed. × ⅔.

(*b*) FOETAL

When casts of foetal ventricles are prepared, some modifications of technique are necessary. The foetal brain is far too delicate to inject with resin after removal from the skull, so it is injected in situ, after removal of the top of the skull.

The brain is fixed by perfusion with 10 per cent formalin via the carotid artery. Then the head is placed in 10 per cent formalin. After twenty four hours, the top of the skull and adjacent dura are removed, and the head is left for two weeks in formalin before the resin injection is made.

FIG. 61

Oblique ventral view of a cast of the lateral ventricles of a 12 weeks' foetus (natural size).

As a very small amount of pressure within the ventricles ruptures the foetal brain, the resin is injected by means of a relatively fine cannula, into comparatively large holes bored into the cerebral hemispheres. The large holes allow the resin to escape freely from both hemispheres at the same time. Double the normal amount of accelerator is added to the resin mixture, to reduce the inhibition of setting. This mixture gels in about twelve minutes at 20°C.

Figure 61 shows a cast of the lateral ventricles of a twelve-weeks' old foetus. When the ventricles of foetal brains between the ages of twelve and sixteen weeks are injected with resin, usually only a cast of the lateral ventricles is obtained, though in exceptional cases the third and fourth ventricles are also filled.

3. A SOLID MODEL OF THE CEREBRAL VENTRICLES

A wax model of the ventricles is first constructed in No. 4 toughened wax, by the procedure described in Chapter 20. The model is constructed in three pieces, consisting of the left lateral ventricle, the right lateral ventricle, and the third and fourth ventricles. A length of wire is incorporated in the aqueduct to strengthen it.

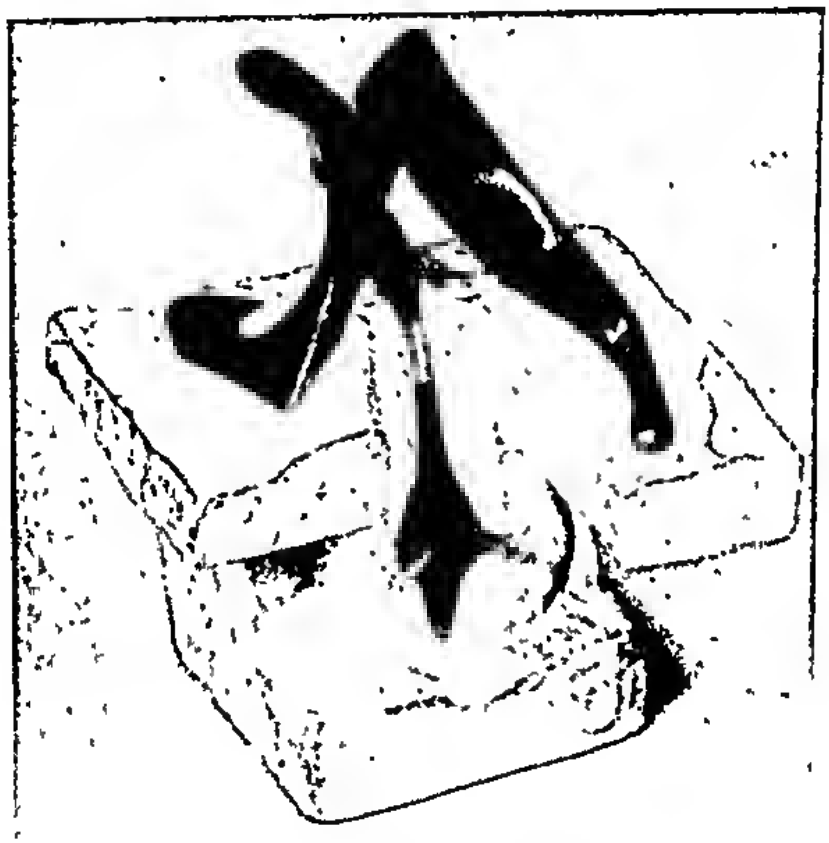


FIG. 62

Plaster stand which holds the three pieces of a wax or resin cast of the cerebral ventricles in their correct relative positions, while the separate parts are being joined. The three parts of a wax model of the ventricles are shown on the stand.

When the three pieces have been more or less completed, they are temporarily stuck together by welding them to a piece of wax placed between the lateral ventricles. Then the two lateral ventricles are bent and trimmed so that the desired degree of symmetry is obtained.

After the model has been completed, a plaster stand is constructed of Dentruset plaster, on which the three pieces rest (see Fig. 62). The stand facilitates the assembly either of wax or resin casts of the three pieces of the model, when replicas are later cast from negative moulds. The stand is made

in the following way. A little tray is constructed of cardboard and Sellotape, and the inside vaselined. Plaster of thick consistency is poured into the tray, and the model is gently pressed into the plaster, so that an impression is formed of the inferior surface of the temporal horns and the third ventricle. Any plaster which flows over the roof of the temporal horns is scraped away before it sets. When the first piece of plaster is hard, the cardboard is removed from around it, and the part of the stand which supports the fourth ventricle is constructed in a similar way. If necessary, a base can be added to increase the stability of the stand. When the stand has been completed, the three parts of the wax model are separated, so that plaster negative moulds can be made of each.

Kaffir D plaster is recommended for making the moulds, as it is harder than Dentruset and consequently a larger number of models can be cast from moulds made with Kaffir D than from those made with Dentruset, before the moulds become unserviceable. Full details concerning the method by which plaster negative moulds are made are given in Chapter 20. Figure 63 shows wax models of the three pieces of the ventricles, and Figure 64 shows the plaster negative moulds. The latter figure indicates the most satisfactory way of constructing the moulds in three pieces without obtaining undercuts.

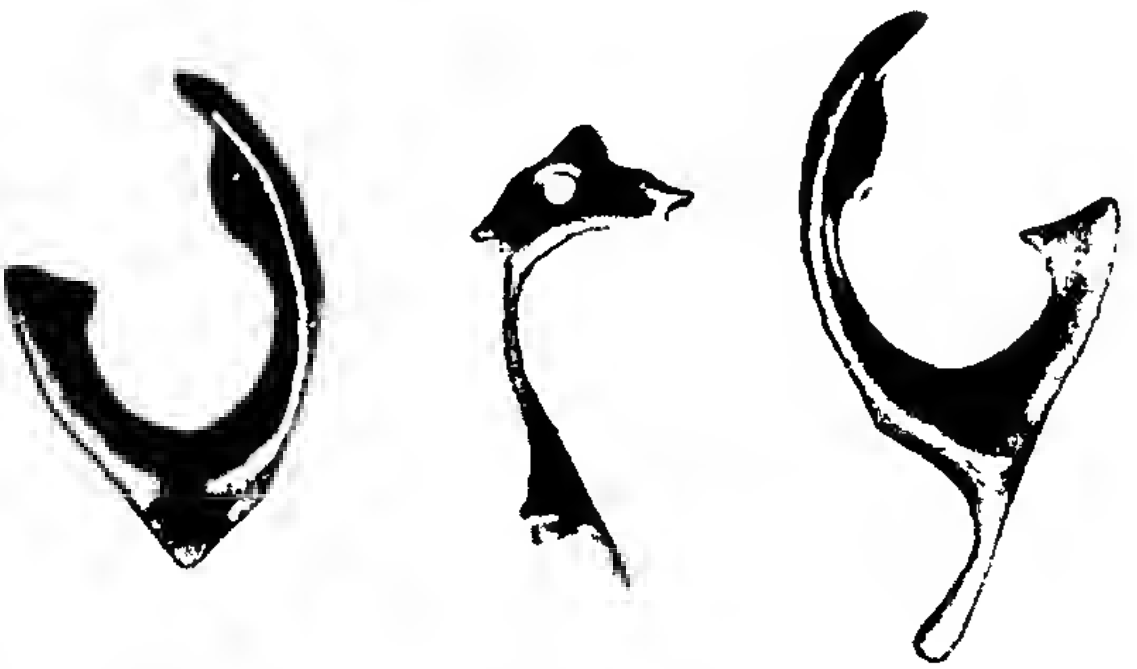


Fig. 63

Wax model of the cerebral ventricles, constructed in three pieces. × $\frac{1}{1}$.

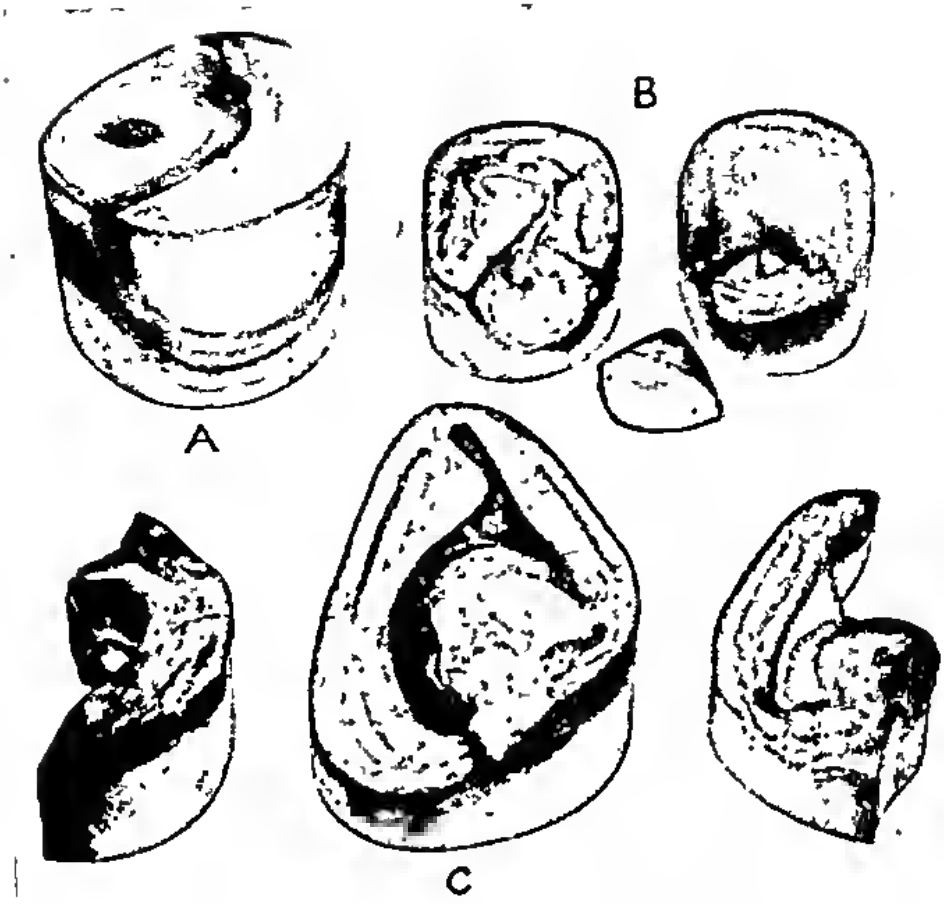


FIG. 64

Plaster negative moulds of the three parts of the model of the cerebral ventricles shown in Fig. 63. A shows the three parts of the mould for the left lateral ventricle assembled. B shows the separated parts of the mould for the third and fourth ventricles, and C those for the right lateral ventricle

Resin casts are made from the negative moulds by the procedure described in Chapter 20. It is advisable to strengthen the cast of the aqueduct by incorporating a piece of wire in it. The three pieces are assembled by placing them on the stand, and by cementing them together with resin cement. Figure 65 shows a completed resin model of the cerebral ventricles. It was modelled from the cast illustrated in Figure 58 (p. 182).

4. A HOLLOW MODEL OF THE CEREBRAL VENTRICLES

This is made by immersing a wax model of the cerebral ventricles in a rectangular container of resin. After the resin has polymerised, the wax is removed by heating the resin block so that the wax melts and can be run out. The rectangular block of resin is then trimmed and polished. The hollow model is partially filled with coloured fluid, to represent the residual cerebro-spinal fluid present in the ventricles when ventriculograms are taken.

FIG. 65

Completed resin cast reproduced from a wax model of the cerebral ventricles. The thickness of the aqueduct is slightly exaggerated to reduce the risk of it breaking. × ⅔. The model was copied from the cast illustrated in Fig. 58. (p. 182)

There are two advantages of constructing a hollow model of the ventricles within a rectangular block of resin. Unlike hollow models which have been made of glass, this model is very strong; and the rectangular block serves to orientate the ventricles.

A wax reproduction of the original wax model of the cerebral ventricles is made by means of the plaster negative moulds used for making the resin cast illustrated in Figure 65.

The plaster moulds are prepared by giving them two coats of a 20 per cent solution of shellac dissolved in spirit. When the shellac is quite hard, it is covered with a thin film of glycerine, which acts as a separating medium. Two or three drops of 20 per cent Manoxol O.T. are added to 25 ml. glycerine, before the latter is applied.

A length of aluminium wire (see Appendix) is placed in the negative mould of the third and fourth ventricles, to reinforce the wax cast of the

aqueduct. Aluminium wire is used in preference to other types because it can be readily dissolved by hydrochloric acid, which does not attack the resin.

The moulds are used at room temperature. No. 4 toughened wax, which melts at 59°C, is heated to the minimum temperature above its melting point which ensures that each mould is completely filled before the wax solidifies. Excessive heating of the wax may cause damage to the moulds. If the room temperature is 20°C the wax is heated to 80°C.

It is not necessary to take any precautions to prevent wax escaping from between the pieces of each mould, as it cools and sets before it reaches the outside. But in the case of the mould of the third and fourth ventricles, it is advisable to warm the aluminium wire slightly in the bunsen flame, immediately before filling the mould with wax, to avoid the risk of the wax setting in the aqueduct before the mould is completely filled.

A few minutes after the moulds have been filled, they are taken to pieces and the wax casts removed. This should be done while the wax is still sufficiently warm to be quite flexible, to facilitate the separation of the wax casts from the columns of wax in the filling and air escape holes of the moulds. The wax remaining in the latter is dislodged by means of a wire ramrod when quite cold. Provided that the inside of each hole is coated with glycerine before the mould is used, the wax is easily removed. It does not matter if the wax casts are bent while being removed, as they are straightened out later.

When molten wax sets it shrinks considerably. As most of the shrinkage is localised in the part of the mould in which the wax solidified last, it is sufficiently noticeable to necessitate reinforcement of some part of the cast with additional wax. The wax casts are washed to remove glycerine from their surfaces and then softened by immersion in water at 45°C. When quite flexible they are replaced in the moulds. The wax casts are carefully inspected by removing one piece of each mould at a time, in order to observe where the most extensive shrinkage has taken place. Wax is welded to these areas and, after softening the casts by immersion in warm water, they are replaced in the moulds and the pieces pressed firmly together. In this way the wax added is pressed into the form of the mould. Surplus wax is forced away from the cast into the slight gap between the individual parts of the mould.

After this treatment has been completed, the wax casts are left in the moulds until quite cold. Then they are removed, and their surfaces smoothed by scraping away surplus wax which is present at the lines of junction of the pieces of the moulds. Finally the casts are polished with a wad of cotton wool soaked in benzene.

The three pieces of the wax model are placed on the plaster stand illustrated in Figure 62, so that they are held in approximately their correct position. Minor adjustments of position can be made with the aid of small lumps of Plasticine. For this technique the distance between the lateral ventricles must be slightly exaggerated, to ensure that a strong resin septum is produced.

The casts of the interventricular foramina are welded to the cast of the third ventricle. This must be done very carefully, to ensure that no air is trapped within the joints. The thickness of the casts of the foramina should be somewhat exaggerated, not only to increase the strength of the wax model, but to facilitate the flow of the coloured fluid later placed in the hollow model.

Next an H-piece, made by soldering three pieces of wire together, is welded in a vertical position into the roofs of the two lateral ventricles. A small knob of solder is applied to the end of each wire before it is welded to the wax model, to make it impossible for the wires to slide out. Then the lower arms of the H-piece are coated with wax. The wax coat must be sufficient to ensure that the knobs of solder do not later prevent the withdrawal of the H-piece from the solid block of resin in which the wax model is embedded. The application of an even coat of wax to the arms of the H-piece is facilitated if the plaster stand on which the model rests is tilted so that the arms of the H-piece are horizontal. Melted wax is transferred to the H-piece on the blade of the scalpel and then smoothed, after it has solidified, by stroking with a heated scalpel blade. Care is needed to ensure that the ends of the H-piece are perfectly embedded in the wax cast. It is advisable finally to melt the wax round each end of the H-piece with the heated end of a needle held on a suitable handle (see p. 42) to allow any air to escape and be replaced by wax. The same precaution should be taken with the joints between the casts of the interventricular foramina and the third ventricle.

Figure 66 shows, natural size, the completed wax model of the cerebral ventricles with the H-piece attached. The H-piece serves the double purpose of providing a convenient means of suspending the wax model in the resin, and also of strengthening the model. The model used for this technique must be sufficiently small to fit into a container of cross section 4 x 4¼ inches, with a margin of approximately ¼ inch all round to allow for trimming of the resin block. If it is attempted to cast a block of much greater cross section than this, it is very difficult to prevent the resin from overheating during polymerisation.

Before the wax model is immersed in the resin mixture, it must be

protected by covering it with a film of some substance insoluble in the resin. A suitable type of polyvinyl alcohol such as Mowiol 50-88 is suitable for this work.

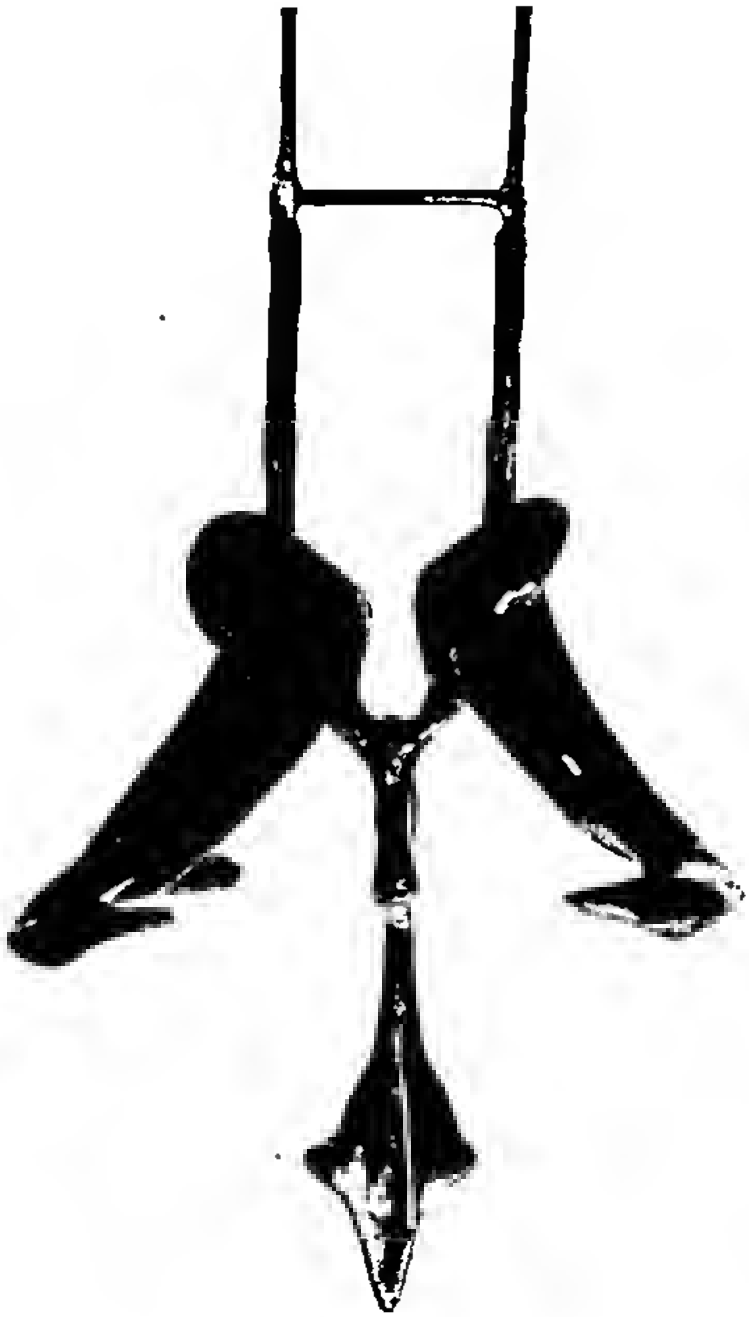


FIG 66

Wax model of the cerebral ventricles, to which a wire H piece has been fixed (natural size) The lower arms of the H piece have been coated with wax The H piece is used to suspend the model in a rectangular container filled with resin

1500 ml. of the Mowiol solution described on page 170, are prepared for coating the wax model of the ventricles. This solution keeps indefinitely and so can be used many times.

The model is dipped in a beaker of the Mowiol solution and, after the surplus has been allowed to drain off, is dried in a draught of cold air from a hair drier. While drying, the model is rotated to ensure an even film. If it appears that the Mowiol is pulling away in places from the wax, it is spread with a sable water-colour brush. Surplus Mowiol which collects in some areas is removed with the brush as long as it remains fluid.

The first coat of Mowiol is left for forty-eight hours to harden and then two more coats are applied in the same way. As the Mowiol is rendered partly insoluble by the resin, it is desirable to apply a relatively thin film, as the insoluble remains of this can be completely dislodged from the hollow model. It may be impossible to remove all traces of a relatively thick film. However, as the wax is rendered quite insoluble by the resin, it is essential to the success of this technique that the film is perfect. It is therefore better to produce a model slightly disfigured by the insoluble remains of the Mowiol, than to risk total failure due to an imperfect film. The wax model should be left for at least a week after the third coat of Mowiol has been applied, so that the protective film is completely hardened before the model is immersed in resin.

A suitable rectangular container to hold the resin is made by soldering pieces of 20 gauge tinned plate together. The design and method of construction of the container are described on page 129, and illustrated in Figure 31 A. The maximum size of container used for this work should not exceed 4 x 4½ inches in cross section. The container should be about 6 inches deep. The inside is painted with a 20 per cent solution of shellac dissolved in spirit, which acts as a separating medium.

The model is suspended in the resin, by passing the two upper arms of the H-piece through holes drilled in a strip of Perspex, which rests across the sides of the container. A lump of Plasticine is placed on the upper surface of the Perspex strip, and pressed around the arms of the H-piece to hold the latter in the desired position. The model is orientated by suspending it in a transparent Perspex box exactly the same size as the metal container. This allows the model to be viewed from the sides. If necessary the arms of the H-piece are adjusted in the Plasticine.

When the model has been centred in the tin container, the position of the Perspex strip is fixed by sticking each end of it to the sides of the container

with pieces of Sellotape. Figure 67 shows the model fixed in position in the tin container.

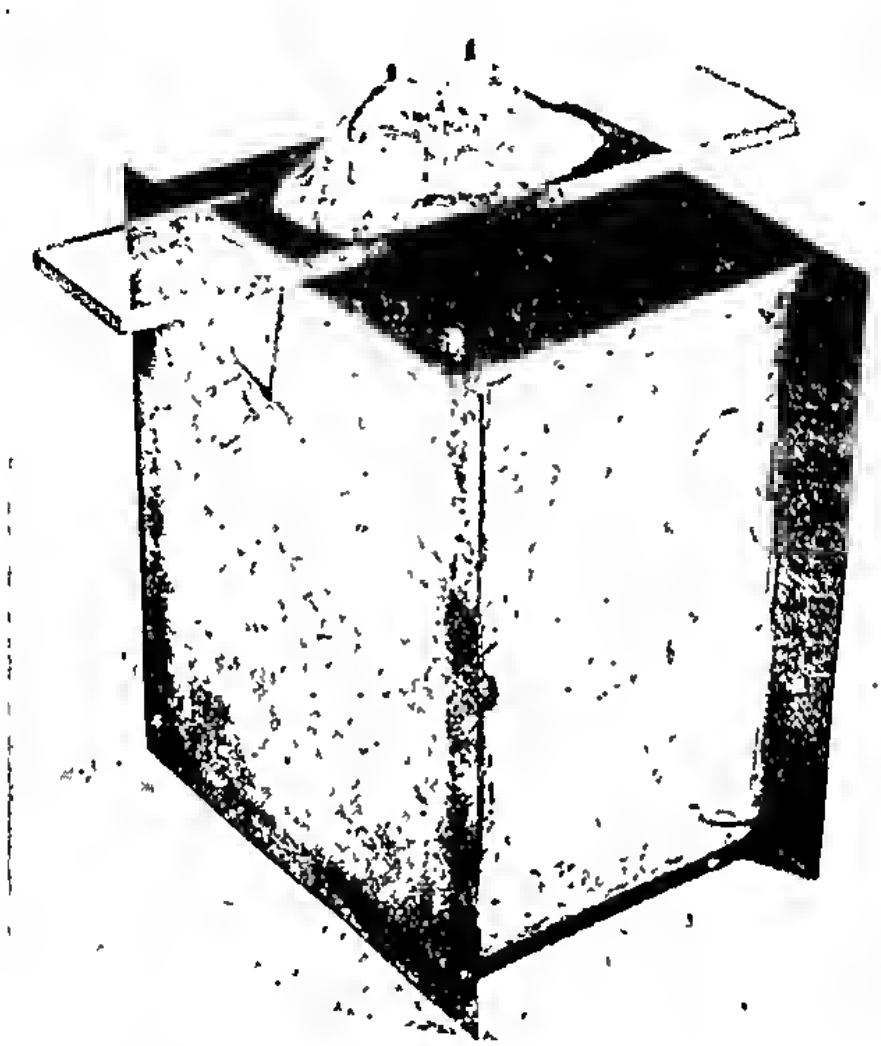


FIG 67

Photograph of the tin container, with the wax model of the cerebral ventricles suspended in it

Using a container of the dimensions given above, 17 units of the following resin mixture are required :

Marco resin 26 C	100 g.
Monomer C	20 g.
Catalyst H C H	0.3 g.
Accelerator E	0.2 ml.

A number of one-foot lengths of glass tube of internal diameter varying from 3-5 mm. are bent so that they are U-shaped, and filled with some of the

resin mixture. When the resin in these tubes has gelled and become fully hardened, they are immersed in boiling water for five minutes, and then the resin rods are removed by cracking the glass. Immersion of the tubes in boiling water facilitates the detachment of the broken glass from the resin. Short lengths of the rods are later used for sealing holes in the rectangular block of resin.

The bulk of the resin mixture is left for forty-eight hours in the beaker in which it is prepared, at a room temperature of 20°C, and protected from light. It is then poured into the rectangular container, which is subsequently kept in a water bath. The temperature of the water is maintained for the next twenty-four hours at 20°C. After this period the water is maintained at 20°C during the day, but cooled to 16°C or lower at night, until the resin gels.

The reason for delaying the transfer of the resin to the container until forty-eight hours after the accelerator has been added, is to reduce to a minimum the period during which the model is immersed in resin before it gels. This procedure reduces the effect which the resin has of rendering the protective film of polyvinyl alcohol partly insoluble. It also reduces the extent to which the resin would soak into the wax, if some tiny defect were present in the protective film.

When the model has been immersed in the resin for twenty-four hours it sometimes becomes covered with small bubbles of unknown origin, which cling tenaciously to its surface. These are dislodged by raising the model out of the resin and allowing the resin clinging to its surface to drain off before it is replaced. When the bubbles have been dislodged from the surface of the model they rise to the surface of the resin within a few hours.

If the resin begins to gel while its temperature is 20°C, the container is placed in a bath of ice water which is placed in the refrigerator overnight, so that the resin is thoroughly cooled. Then the temperature of the water bath is maintained at 20°C by day and 16°C or lower by night for three days, to guard against the risk of overheating. During the day the resin is occasionally inspected and, if it begins to heat up, it is immediately cooled by placing the container in ice water.

If the temperature of both the resin and the water bath is 16°C or lower when gelling commences, it is not necessary to cool the resin in the refrigerator, provided that the temperature of the water bath can be maintained at 16°C or lower for the next twenty-four hours.

Although it is not advisable to undertake this technique when the room temperature is likely to exceed 20°C during any part of the day, owing to

the difficulty of preventing the heat-producing phase of polymerisation from getting out of control, it is possible to do it successfully provided the operator has sufficient experience to recognise the signs (lightening of colour and increased viscosity) which indicate that gelling is imminent. But it is essential that the temperature of the resin is not higher than 20°C at the onset of the heat-producing phase of gelling, and that the resin is effectively cooled immediately it starts to generate heat.

In order to observe clearly the colour change which immediately precedes gelling, a sample of resin is poured into a test tube when the container is filled. The test tube is kept at all times in the water bath which is used to cool the container, and is also protected from daylight. Thus the resin in the test tube polymerises under the same conditions as that in the metal container. The yellow colour of the resin in the test tube becomes very much paler at least an hour before the heat-producing phase of polymerisation commences; in fact a gradual colour change commences several hours earlier. Thus the sample in the test tube provides a valuable indication, especially under difficult temperature conditions, of the approach of the heat-producing phase of polymerisation. A further test tube containing a sample of the same resin mixture should be kept in the refrigerator. The low temperature at which this sample is stored almost completely arrests polymerisation, so it provides a useful colour change indicator.

After the resin has gelled, it is left for a further three days at room temperature. Then the container is placed in an oven at 45°C for two weeks to accelerate the hardening of the resin, which would otherwise take a very long time owing to the small amount of catalyst and accelerator in the mixture.

Next the tin container is removed from around the block by rotting the solder with mercury, by the method described on page 131, and illustrated in Figure 31 B.

The H-piece is removed by pulling it out while the block is warm and the wax consequently quite soft. The holes left in the resin by the H-piece are drilled out so that they have walls of even diameter, and can later be plugged with resin rods. A small hole is drilled in the base of the block into the fourth ventricle, to facilitate the removal of the wax, and subsequent washing of the cavities of the hollow ventricles.

The block is then placed in an oven at 75°C overnight, to melt the wax. The block must not be heated above this temperature, as this would render the polyvinyl alcohol completely insoluble, so that it could not be washed out.

As much as possible of the wax is shaken out of the resin block, and the

rest washed out with hot benzene. Considerable patience is required to remove all the wax as, in spite of all the precautions taken, it is rendered rather less soluble that it originally was. Sometimes the last traces of wax in the temporal horns can be removed with hot xylol more quickly than with benzene.

When all the wax has been removed, the hollow ventricles are washed out thoroughly with spirit, to remove the last traces of the benzene. Then the polyvinyl alcohol is washed out with hot water.

The aluminium wire is dissolved by running a steady stream of 50 per cent hydrochloric acid into one of the holes in the top of the block, and allowing it to escape via the hole in the bottom.

If the hollow ventricles are *not* perfectly clean, they are washed out alternately with spirit, benzene, spirit and water, until no further improvement in their appearance is observed. It is necessary at this stage to polish the walls of the block somewhat, in order to observe the ventricles clearly.

Any remains of the original protective film which still adhere to the walls of the ventricles are removed in the following way. The block is heated to 65°C and the cavities filled with benzene and mercury. The holes are closed with rubber bungs and the block is shaken very vigorously. The mercury dislodges the remains of the film. This treatment is repeated several times with hot spirit and mercury.

Next the cavity of the ventricles is washed out with distilled water, and very thoroughly dried with the assistance of a hair drier, which can be clamped in position so that hot air plays on the holes in the top of the block for several hours.

When the inside is quite dry, resin rods, which have been rubbed with abrasive paper until they fit firmly but not tightly into the two holes in the upper surface of the block, are cemented into the holes with resin cement of the following composition :

Marco resin 28 C	100 g.
Monomer C	10 g.
Catalyst H C H	6 g.
Accelerator E	approx. 6 ml.

One unit of this cement is prepared, but without the addition of the accelerator. When cement is required a few ml. of the mixture are transferred to a porcelain palette, and two or three drops of accelerator added and stirred in immediately before use. A bulky mass of this cement heats up so much

manipulated by rotating it, and partially withdrawing and reinserting it, to dislodge the air. If necessary the rod is withdrawn completely and, after being coated with more cement, inserted again. If the rods fitted loosely instead of firmly into the holes, unsatisfactory joints are always produced, as a result of shrinkage of the cement during setting.

The block is left for forty-eight hours, while the cement hardens, before the projecting ends of the two rods are sawn off. Then the hollow ventricles are partly filled with a solution of Light Green dye dissolved in distilled water containing 10 per cent formalin. A sufficient number of drops of 20 per cent Manoxol O.T. are added to this fluid so that it wets the walls of the ventricles instead of clinging in droplets. It is important not to add even one drop too much of the Manoxol, as this causes the green fluid to be converted to a frothy mass of bubbles when the resin block is manipulated. The intensity of the green should be such that the fluid level is clear, but not so intense that the film of fluid clinging to the walls of the hollow ventricles also looks noticeably green.

The hole in the base of the block is sealed in the same way as the two holes in the top. The walls of the hole are first dried either with a pipe cleaner or small wads of cotton wool held in iris forceps.

After leaving the block for forty-eight hours for the cement used to fix the rod into the base to harden, the block is trimmed, smoothed and polished, by the method described in Chapter 16. Figure 68 shows a photograph of a hollow model of the cerebral ventricles prepared by this method.

Chapter 22

CASTS FROM TEMPORAL BONES

I. INTRODUCTION

IN order to obtain a complete cast of the cavities of a temporal bone, the tissues must be macerated very thoroughly, and the cavities well washed out, to remove the debris produced by maceration. But the method of maceration must not be such as to make the bone too porous, as otherwise the resin soaks into the bone itself so extensively that it is impossible to isolate the cast of the cavities from the resin-impregnated bone without damage.

In devising a satisfactory technique two problems must be solved. The first is to fill the cavities completely. This task is complicated by the fact that many of these are slender culs-de-sac normally filled with air in the dry macerated bone. The second problem is to prevent the resin in the cavities from running out via the numerous apertures on the surface of the bone, before the resin gels.

If the technique is skilfully applied, an almost perfect cast is invariably obtained. But, as the cavities originally occupied by blood vessels and the cancellous parts of the bone are unavoidably filled with resin at the same time, extensive pruning of the cast is necessary.

If a cast of the cavities alone is being prepared, a somewhat incomplete temporal bone is quite adequate; but when a cast of the cavities made in coloured resin is embedded in a transparent cast of the original bone, a bone is required with the whole of the squama and at least part of the zygoma, as the presence of these two parts greatly facilitates orientation of the cast by students.

The method employed in the latter technique is basically simple. The cavities of the bone are filled with coloured resin. After being encased in plaster, the bone is dissolved in acid, and the cavity left in the plaster is filled with transparent resin. Then the plaster is chipped away from the transparent cast. Although the actual procedure is more elaborate than this brief outline suggests, the only real problem involved is to devise a reliable method of ensuring that the cast of the cavities is embedded in the correct position in the transparent cast of the bone.

2. CASTS OF THE CAVITIES

Temporal bones removed at post mortem are required for this technique. The bone must not have been placed in any kind of fixative. All flesh is removed from the bone, and the periosteum carefully stripped off. Special care is needed when removing the periosteum from the middle fossa, as this part of the bone is often fragile.

The bone is macerated by placing it in a large volume of water for six months. The water tank must be kept in a dark place to prevent algae growing on the bone and even inside it. The water should be maintained at about 30°C, but provided that the temperature does not fall below 20°C satisfactory maceration results, although it may take longer. A tiny thermostatically-controlled immersion heater, such as is used to maintain the temperature of small domestic aquaria containing tropical fish, is ideal for maintaining the temperature of the macerating tank.

After about three months in water, the bone is removed, and its surface scrubbed. Water is squirted into the principal apertures, and the bone shaken to remove as much as possible of the water filling the cavities of the mastoid air cells. The treatment is repeated several times, and any adipocere (an insoluble waxy substance resulting from decomposition of fat) on the surface of the bone is scraped away. Then the bone is replaced in clean water and left for a further three months. After this period, it is removed, cleaned and rinsed, and a 1/16 inch diameter hole is drilled into the lateral wall of the mastoid process so that, if the air cells extend into it, air can escape when the cavities are being filled with resin. Then the bone is thoroughly dried.

The dried bone is impregnated in vacuo with a 5 per cent solution of Mowiol 50-88, to which 1 per cent of 20 per cent Manoxol O.T. solution and 2 per cent formalin have been added. The Mowiol, which is a form of polyvinyl alcohol, protects the bone from subsequent impregnation by the resin. The Manoxol is a very powerful wetting agent and facilitates the absorption of the Mowiol by the bone (see p. 170 for details concerning the preparation of a solution of Mowiol).

After removal from the Mowiol solution the bone is shaken vigorously to remove as much as possible of the fluid in its cavities. It is then dried in a current of warm air from a hair drier, being rotated continuously while it is drying to prevent pools of solution accumulating in the cavities. Such pools would leave deposits of Mowiol which might obstruct the finer cavities. The drying must be continued for an hour, with intermittent shaking, to ensure that no liquid remains within the bone.

One end of a straight 3 inch length of glass tubing, of internal diameter approximately the same as that of the external auditory meatus, is ground so that it fits closely against the bone around the meatus in a vertical position, when the bone rests on the table with the meatus uppermost. The glass

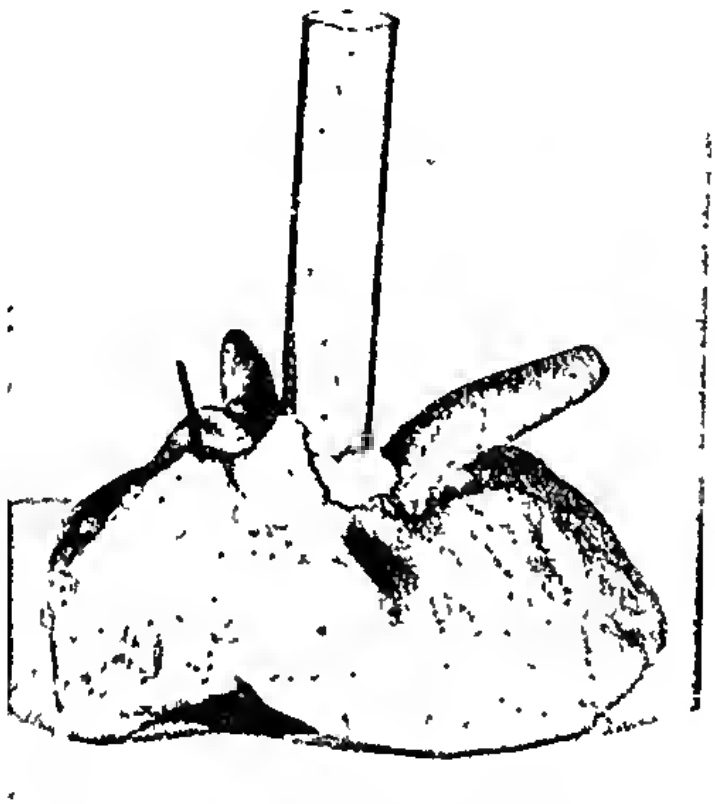


FIG 69

Photograph of a temporal bone prepared for filling the cavities with resin. A glass tube is fixed to the external auditory meatus, to serve as a reservoir for the resin, and the bone is completely encased in Plasticine, to prevent leakage of resin from its surface. A piece of wire has been placed in an escape hole drilled into the mastoid air cells, to prevent the hole becoming choked with Plasticine × ⅔

tube is cemented to the meatus with Dentruset plaster. It forms a reservoir from which the coloured resin, used to fill the cavities of the bone, is run in.

A piece of Sellotape is stuck over the opening of the internal auditory meatus, and a piece of wire is inserted into the escape hole in the mastoid process. Then the carotid canal is packed with Plasticine, and the whole bone encased in Plasticine, to prevent the escape of resin when the cavities are being filled. Care is needed when the Plasticine is applied to the bone, to avoid forcing it into the Eustachian tube, and the opening of the facial nerve canal. Figure 69 shows a temporal bone prepared in this way.

A resin mixture of moderately low viscosity with a working life of about 1½ hours is required for filling the cavities. The pigment used to colour the resin must have a high resistence to hydrochloric acid, as the bone is not completely dissolved until it has been in concentrated acid for about three weeks. Red lake pigment powder M.11 is recommended. It must be mixed with the resin with exceptional care, and the resin mixture must be allowed to stand for at least two hours before being decanted from the beaker in which it was prepared, to ensure that any large particles of pigment powder are removed from the mixture before the accelerator is added. Even comparatively small lumps of pigment powder might obstruct some of the tiny channels within the bone which are to be filled with resin.

If the working temperature is 20°C, the following mixture is recommended :

Marco resin 26 C	100 g.
Monomer C	20 g.
Catalyst H C H	2 g.
Red lake pigment powder M. 11	1 g.
Accelerator E	1 ml.

A little of the resin mixture is placed in the refrigerator for future use before the accelerator is added. After the accelerator has been incorporated the mixture is allowed to stand for ten minutes while air bubbles come to the surface. Then some of it is poured into the glass tube (see Fig. 69), and the bone is rocked as much as possible without spilling the resin. This allows a good deal of the air trapped within the cavities to escape and be replaced by resin. Then the wire is removed from the escape hole in the mastoid process and the bone again rocked. When resin free from air bubbles escapes from this hole, it is closed by pinching the Plasticine together.

The bone is next placed in a vacuum chamber with the reservoir half full of resin, and subjected to a water vacuum for fifteen minutes. During this period the vacuum is reduced slightly whenever the resin bubbles so vigorously that it begins to overflow from the top of the glass tube. Then pressure is restored to normal and, if necessary, more resin is added to the reservoir.

The vacuum treatment is repeated twice and then the glass tube is completely filled with resin. A finger, protected by a rubber finger stall, is

placed over the open end of the tube, and the bone is slowly rotated in all planes, until the resin gels. It should also be swung vigorously from time to time. This procedure ensures that if any air still remains in the cavities of the bone, most of it is enclosed within the resin cast, instead of causing an incomplete cast to be formed.

Twenty-four hours after the resin has gelled, the Plasticine is removed from the bone, the last traces being wiped away by immersing the bone in benzene and brushing it with a fairly stiff brush. Any resin which has filled spaces between the surface of the bone and the Plasticine is removed with a scalpel. This is facilitated by dipping the bone into boiling water for a few seconds to soften the resin. The plaster by which the glass tube is attached to the external auditory meatus is chipped away with bone forceps, and the tube removed from the stalk of resin which it encloses. If any openings of the bone are incompletely filled, the cast is completed by topping up with the resin placed in the refrigerator for this purpose.

The resin stalk projecting from the external auditory meatus is cut through with a burr attached to the flexible drive of a dental lathe, flush with the opening of the meatus. A hole is drilled into the resin within the meatus, into which the mounting rod can later be fixed.

The bone is left for three days for the resin to harden, and then it is washed in running water at about 45°C for a week, to remove as much as possible of the polyvinyl alcohol with which it was impregnated, as the removal of the latter facilitates maceration of the bone in acid. When the bone has dried, it is immersed in concentrated hydrochloric acid, which destroys it completely in about three weeks.

After removal from the acid, the cast is held under water while a gentle stream of cold water is directed on it, to free the cast from acid and debris. Extensive pruning is necessary to remove resin which has formed a cast of the network of blood vessels within the bone. Most of the pruning can be done by teasing away the surplus resin with a mounted needle (see p. 42). Care is needed to preserve the cast of the canal of the chorda tympani, the aqueduct of the cochlea, and the sacculus endolymphaticus. The latter is attached to the rest of the cast by such a tiny stalk that it frequently breaks off. It can however be preserved, and cemented in position later with a drop of transparent resin cement. It is sometimes necessary to use bone forceps to remove resin which has filled some of the air spaces of the cancellous part of the bone. If during this work it is found that any part of the bone is not completely destroyed, the cast must be returned to the acid bath.

Some of the mastoid air cells are usually only partly filled with resin. They can be topped up with a little resin cement of the same colour as the rest of the cast. Any whitish patches on the cast which do not temporarily disappear when the surface is wetted with benzene are removed by scraping. The patches are usually caused by adipocere. If any casts of air cells become detached, they are cemented in their original position with resin cement.

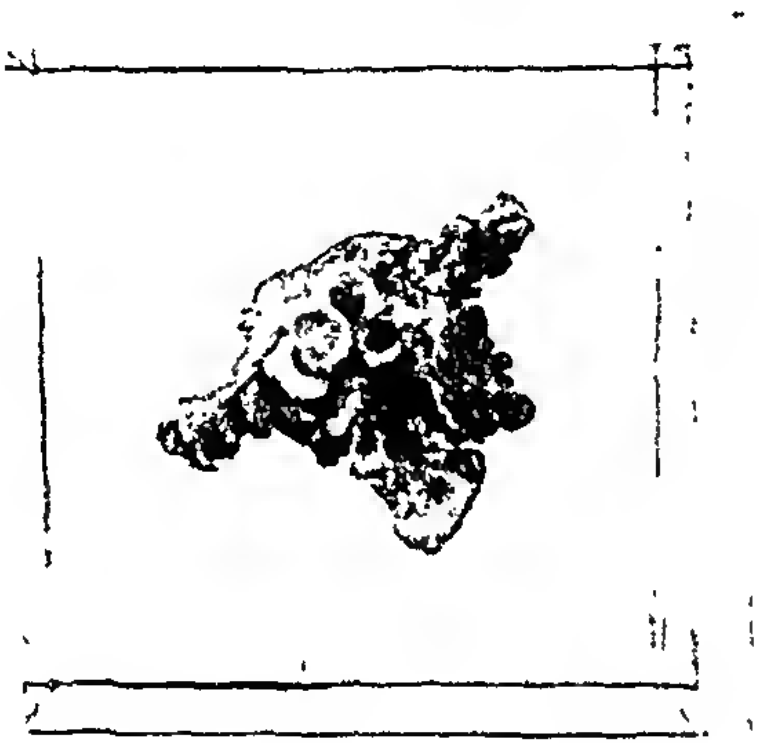


FIG. 70

Medial view of a cast of the cavities of a right temporal bone, embedded in a rectangular block of transparent resin. × ⅔.

A brass wire for mounting is cemented with resin cement into the hole previously drilled in the cast of the external auditory meatus. The cast can be mounted either by enclosing it in a rectangular Perspex box, or by embedding it in a block of transparent resin. The former method allows a much clearer view of the cast but, if the Perspex box is dropped, the cast may be damaged. If the cast is embedded, it is almost indestructable, but the refractive index of the transparent resin causes considerable foreshortening of the view of the cast. Figure 70 shows a cast embedded in a rectangular block of transparent resin, and Figure 71 shows a cast which is mounted in a Perspex box.

If the cast is to be mounted in a Perspex container, it is coated with the following resin mixture :

Marco resin 28 C	100 g.
Monomer C	20 g.
Catalyst H C H	6 g.
Accelerator E	6 ml.

FIG. 71

Medial and somewhat superior view of a cast of the cavities of a right temporal bone, mounted in a rectangular Perspex box. The air cells are more extensive in this specimen than normally. (Natural size)

The cast is dipped in this mixture, and the surplus allowed to drain off. Then the cast is rotated on its wire rod until the resin gels, to ensure that an even film is applied. The film of resin not only improves the general appearance of the cast, but strengthens it, by sticking the casts of the air cells, some of which are attached only by slender stalks, firmly to the rest of the cast. The resin mixture sets with a tack-free surface.

The end of the mounting wire is fixed to one of the sides of the Perspex container by drilling a $\frac{3}{16}$ inch hole through the latter. Then a short length of $\frac{3}{16}$ inch diameter Perspex rod is cemented into the hole with Tensol No. 6 cement. The brass mounting wire is cemented with Tensol No. 6 cement into a hole drilled in the centre of the Perspex rod. The full details concerning

methods of constructing rectangular Perspex containers are given in Chapter 8, and the method of sticking on the tops of such containers, which requires certain precautions, is described in Chapter 9.

If the cast is to be embedded in a solid block of transparent resin, it is coated with a Marco resin 26 C mixture, instead of 28 C, so that the surface of the coated cast is permanently tacky. This prevents the transparent resin pulling away from the cast. Full details concerning the embedding of specimens in rectangular blocks are given in Chapter 17.

3. A COLOURED CAST OF THE CAVITIES EMBEDDED IN A TRANSPARENT CAST OF THE ORIGINAL BONE

The first half of this technique is exactly the same as the preceding one, but after the cavities of the bone have been filled with coloured resin, the resin stalk projecting from the external auditory meatus is not cut off.

The bone is encased in three pieces of Dentruset plaster, the largest surrounding the resin stalk, while the other two fit into this piece by means of registration slots. When deciding the exact extent of each of the three pieces, it must be borne in mind that subsequently it will be necessary, after most of the bone has been destroyed by acid, to remove the two pieces of plaster adjacent to the ridge of the petrous bone, and then to remove the cast of the cavities from the remaining piece of plaster, by sliding the resin stalk through the plaster in which it is embedded. If an unhappy choice is made of the boundaries of the three pieces of plaster, it may not be possible to remove the cast of the cavities without breaking some part of the plaster. However, if a small piece of plaster is broken off, it should be preserved, as it can be stuck into position again at a later stage with Seccotine.

It is necessary to make the plaster cast of the carotid canal separately, as the cast of the cavities cannot be removed from the base of the plaster mould without removing the cast of the carotid canal at the same time. The canal is filled with Dentruset plaster and, when this has set, the exposed surface of the plaster is trimmed and coated with vaseline, which acts as a separating medium.

Next the bone is placed on a sheet of glass with the resin stalk projecting upwards. A receptacle into which plaster can be poured to cover the exposed surface of the bone is made in the following way. A rim of Plasticine about three-quarters of an inch wide is built up all round the margin of the bone. Then a vertical Plasticine wall is built round the outside of the rim, extending an inch above the highest part of the bone. The resin stalk is coated with

vaseline, and the surface of the bone and the Plasticine are painted with water to which a sufficient number of drops of 20 per cent Teepol O.T. have been added so that the water forms an even film.

Dentruset plaster, prepared by mixing 100 parts by [illegible] of plaster

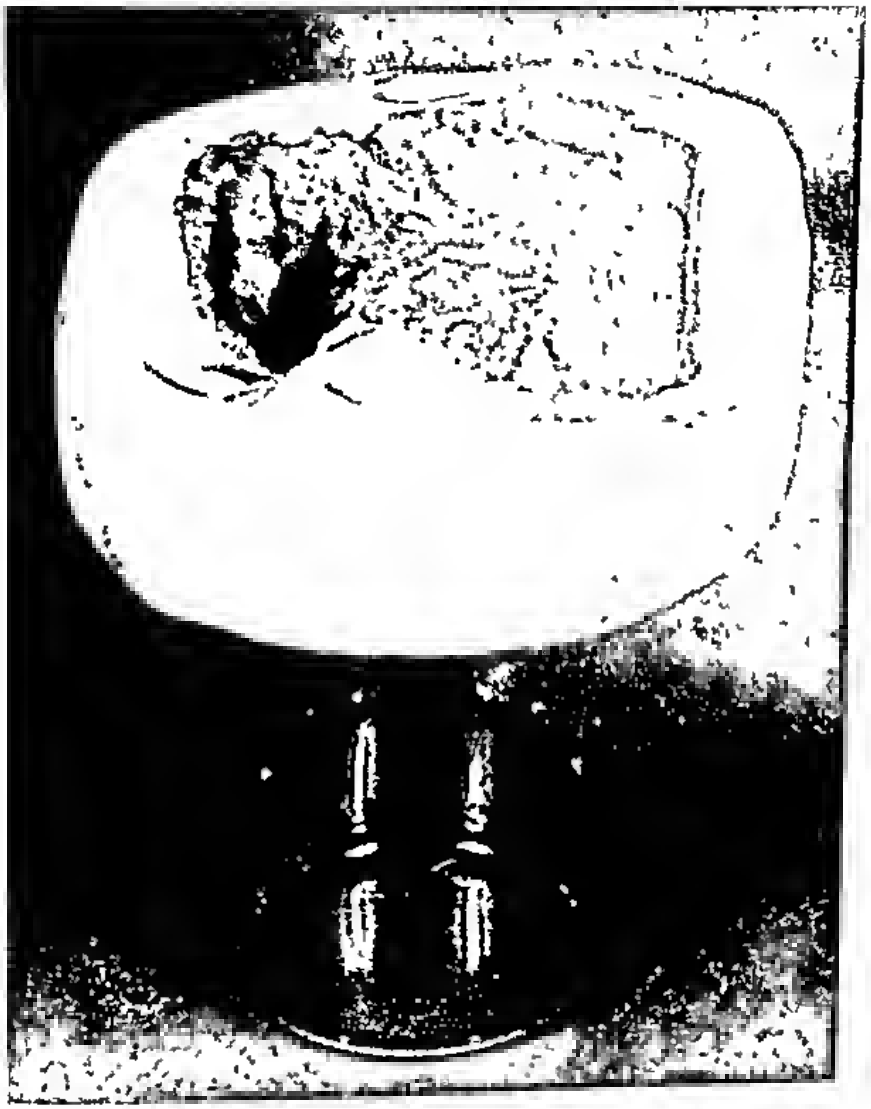


FIG. 72

Photograph of a bone, after the first stage of encasing it in plaster has been completed. Note the resin stalk projecting through the base of the plaster, which is resting on a beaker. × ⅔.

with 45 parts water, is poured into the receptacle formed by the bone and the Plasticine, until the uppermost part of the bone is covered to a depth of half an inch. The glass base is gently shaken until the plaster sets, to dislodge air in the plaster. It is important that all the plaster is tipped quickly into the water, instead of adding it slowly while stirring, as is sometimes recommended. For the success of this technique it is essential to produce a Dentruset plaster of maximum hardness, which will stand up reasonably well to the severe

methods of constructing rectangular Perspex containers are given in Chapter 8, and the method of sticking on the tops of such containers, which requires certain precautions, is described in Chapter 9.

If the cast is to be embedded in a solid block of transparent resin, it is coated with a Marco resin 26 C mixture, instead of 28 C, so that the surface of the coated cast is permanently tacky. This prevents the transparent resin pulling away from the cast. Full details concerning the embedding of specimens in rectangular blocks are given in Chapter 17.

3. A COLOURED CAST OF THE CAVITIES EMBEDDED IN A TRANSPARENT CAST OF THE ORIGINAL BONE

The first half of this technique is exactly the same as the preceding one, but after the cavities of the bone have been filled with coloured resin, the resin stalk projecting from the external auditory meatus is not cut off.

The bone is encased in three pieces of Dentruset plaster, the largest surrounding the resin stalk, while the other two fit into this piece by means of registration slots. When deciding the exact extent of each of the three pieces, it must be borne in mind that subsequently it will be necessary, after most of the bone has been destroyed by acid, to remove the two pieces of plaster adjacent to the ridge of the petrous bone, and then to remove the cast of the cavities from the remaining piece of plaster, by sliding the resin stalk through the plaster in which it is embedded. If an unhappy choice is made of the boundaries of the three pieces of plaster, it may not be possible to remove the cast of the cavities without breaking some part of the plaster. However, if a small piece of plaster is broken off, it should be preserved, as it can be stuck into position again at a later stage with Seccotine.

It is necessary to make the plaster cast of the carotid canal separately, as the cast of the cavities cannot be removed from the base of the plaster mould without removing the cast of the carotid canal at the same time. The canal is filled with Dentruset plaster and, when this has set, the exposed surface of the plaster is trimmed and coated with vaseline, which acts as a separating medium.

Next the bone is placed on a sheet of glass with the resin stalk projecting upwards. A receptacle into which plaster can be poured to cover the exposed surface of the bone is made in the following way. A rim of Plasticine about three-quarters of an inch wide is built up all round the margin of the bone. Then a vertical Plasticine wall is built round the outside of the rim, extending an inch above the highest part of the bone. The resin stalk is coated with

vaseline, and the surface of the bone and the Plasticine are painted with water to which a sufficient number of drops of 20 per cent Manoxol O.T. have been added so that the water forms an even film.

Dentruset plaster, prepared by mixing 100 parts by weight of plaster

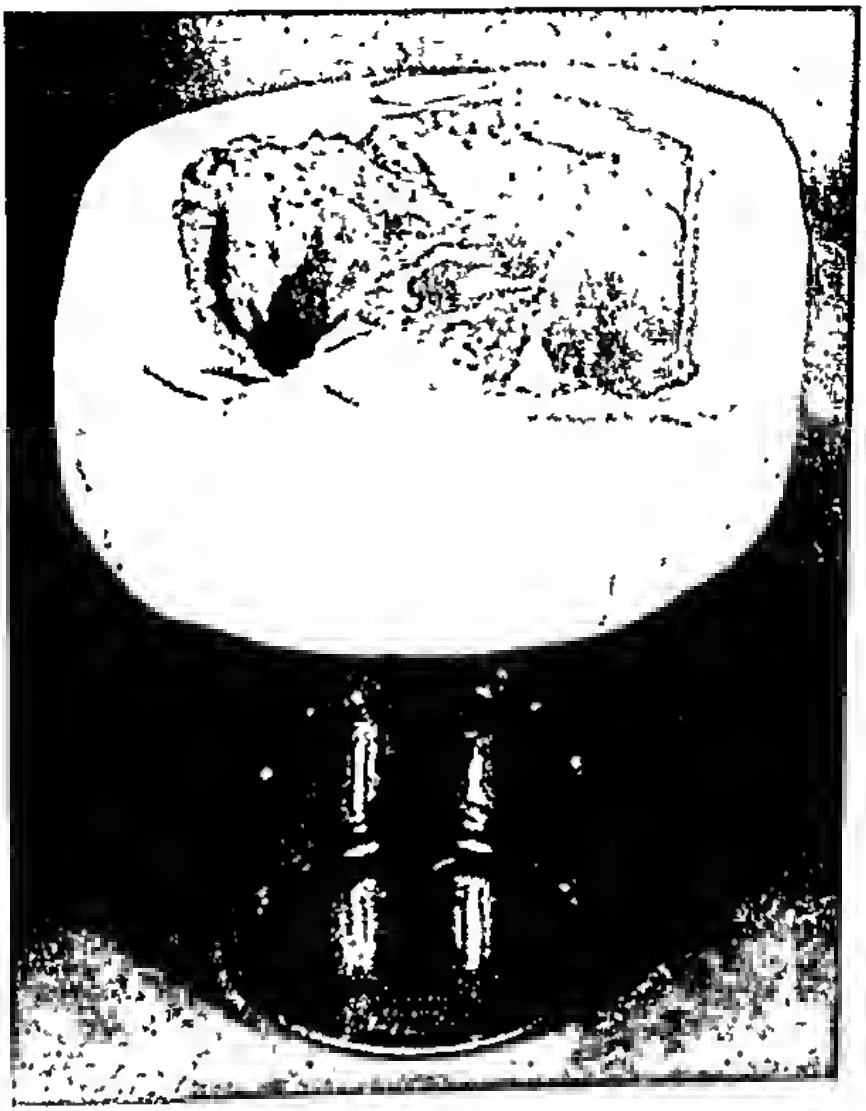


FIG 72

Photograph of a bone, after the first stage of encasing it in plaster has been completed. Note the resin stalk projecting through the base of the plaster, which is resting on a beaker × ⅔

with 45 parts water, is poured into the receptacle formed by the bone and the Plasticine, until the uppermost part of the bone is covered to a depth of half an inch. The glass base is gently shaken until the plaster sets, to dislodge air in the plaster. It is important that all the plaster is tipped quickly into the water, instead of adding it slowly while stirring, as is sometimes recommended. For the success of this technique it is essential to produce a Dentruset plaster of maximum hardness, which will stand up reasonably well to the severe

Fig. 73

Photograph of the plaster mould with one of the upper pieces removed, showing the cast of the cavities in position. Note the filling hole, and two escape holes. × ⅔.

treatment to which it is subsequently subjected, without crumbling. Maximum hardness is achieved only if plaster in perfect condition is used and mixed as recommended above.

After the plaster has set, the Plasticine is removed, the surface of the rim of plaster which now surrounds the bone is trimmed, and register slots are cut in it. The plaster is left until completely dry, and then the surface of the plaster rim is coated with vaseline. Figure 72 shows the bone after this stage of embedding it in plaster has been completed.

By following a similar procedure the bone is completely encased in two more pieces of plaster, the dividing line between them running along the ridge of the petrous bone. If, after the first piece of plaster has dried, the squama of the temporal bone curls up a little, the plaster and bone are soaked in water overnight before the second piece is constructed, as this treatment usually results in the bone lying flat again, and does not harm the plaster, provided that the latter was completely dry before being soaked. If soaked before fully dried, the hardness of the plaster is affected. Figure 73, taken after the bone has been dissolved in acid, gives an idea of the shape of the three pieces.

When the plaster is completely dry, it is immersed in hydrochloric acid, in order to dissolve the bone. Concentrated hydrochloric acid rots plaster very severely, and so it must be diluted by mixing equal parts of concentrated acid and water. The plaster is immersed in the acid for three weeks. Some warping of the plaster takes place, but fortunately this does not materially affect the accuracy of the cast of the bone which is subsequently produced, though rather conspicuous gaps usually appear between the individual pieces of plaster.

The mould is removed from the acid and left overnight in a sink full of cold water, so that some of the acid can soak out of the plaster. It must not be washed in running water, as this corrodes the plaster. Next the two pieces of plaster covering the petrous bone are removed. The resin cast of the cavities, to which some incompletely macerated bone still adheres, is then removed from the remaining piece of plaster by pushing the resin stalk through it. It is usually necessary to rotate the stalk slightly during removal.

The plaster cast of the carotid canal is removed from the partially destroyed bone, and the latter is placed in concentrated hydrochloric acid until all the bone is dissolved. The three pieces of plaster are cleaned by holding them under water and brushing them very gently with a soft brush. They are placed overnight in a large volume of water to remove the bulk

of the acid from them, and then they are dried in an oven at 45°C. They are left in the oven until they no longer smell of hydrochloric acid.

A ¼ inch diameter filling hole is drilled through the piece of plaster which originally covered the posterior fossa (see Fig. 73). The hole is drilled from the inside surface, as a piece of plaster is usually broken off as the drill emerges. The outside of the filling hole is enlarged until it is cone-shaped. Two small escape holes are also drilled through the plaster to allow air to escape when the mould is filled with resin. If the original bone had a very thin squama, the plaster is scraped away from the corresponding part of the inside of the mould, so that a somewhat thicker cast of the squama is produced. This makes the cast less fragile.

Then all surfaces of the mould (including the plaster cast of the carotid canal), with which the resin will come in contact, are given several coats of 20 per cent shellac solution dissolved in spirit, applied at intervals of twenty-four hours until a glossy surface is produced. The shellacked surface is coated with the 15 per cent aqueous solution of Mowiol 50-88 described on page 170. The shellac closes the pores of the plaster, and facilitates the application of the Mowiol, which acts as a separating medium, and facilitates the removal of the plaster from the resin cast. The Mowiol must be really dry before the mould is assembled.

The resin cast of the cavities is pruned and cleaned in the same way as when an isolated cast of the cavities is being prepared. Then it is coated with the following resin mixture :

Marco resin 26 C	100 g.
Monomer C	25 g.
Catalyst H C H	4 g.
Accelerator E	*2 ml.*

This mixture sets with a tacky surface which ensures that the transparent resin in which the cast is to be embedded does not pull away from its surface when it hardens. The cast must be protected from dust after being coated with this mixture.

The stalk of the cast is coated with Seccotine cement, made by mixing equal quantities of Seccotine and Dentruset plaster (Plaster is added to the Seccotine to make a cement with good space-filling properties). Then the stalk is slid into the base of the plaster mould. After the plaster cast of the carotid canal has been cemented into position with Seccotine cement, the two pieces of plaster forming the roof of the mould are placed very carefully, one

at a time, in position, and the interior of the cavity so formed is inspected, in order to check whether the resin cast and the cast of the carotid canal are in exactly the positions they originally occupied. Any adjustments which are necessary are made before the cement has hardened. Figure 73 shows the plaster mould at this stage, with one of the upper pieces removed.

The base of the mould is left overnight while the cement hardens, and then the two pieces of plaster forming the roof are cemented in position with Seccotine cement. The cement holding the first piece to the plaster base is allowed to harden before the second piece is stuck on. A thick layer of Seccotine cement may be required to fill completely the gaps between the pieces of plaster caused by warping.

After the mould has been assembled it is left for twenty-four hours for the cement to harden, before it is filled with resin.

A resin mixture with a working life of about 1½ hours is suitable for filling the cavity of the mould. This gives ample time for manipulations which are necessary to dislodge air trapped inside the mould when it is first filled.

At a working temperature of 20°C the following mixture is suitable :

Marco resin 26 C	100 g.
Monomer C	30 g.
Catalyst H C H	2 g.
Accelerator E	1 ml.

After the accelerator has been incorporated, the mixture is allowed to stand for fifteen minutes while air bubbles come to the surface. Then the mould is filled with resin by the method described on page 172, and illustrated in Figure 52. When the mould is completely filled with resin, fingers, protected by rubber finger-stalls, are placed over the filling and escape holes, and the mould is rotated in all planes to dislodge air trapped under the cast of the cavities. After topping up with resin to replace any air which is removed, the mould is allowed to stand on the table until the resin gels. It should be inspected from time to time, as sometimes more air escapes so that the mould needs to be topped up with resin again.

The mould is left for four days for the resin to harden. Then it is placed in hot running water for forty-eight hours to remove the Seccotine cement, and soften the film of Mowiol, with which the inside of the mould is coated. The plaster is chipped away from the resin cast with bone forceps. The plaster within the carotid canal is excavated with a stout triangular

needle mounted on a handle. Some care is needed to avoid any damage to the resin cast while the plaster is being chipped away.

The surfaces of the cast of the petrous bone, including the mastoid process, are slightly smoothed with the aid of a dental drill, to reduce the distortion of the view of the coloured cast of the cavities, due to the curvature of these surfaces. Then the whole surface of the cast is scoured with pumice powder, to facilitate the adherence of a coat of resin which is applied to make the cast completely transparent.

At this stage the cast is opaque when dry, but fairly transparent when its surface is wet. After being thoroughly washed and dried, the cast is made permanently transparent by coating it with the following resin mixture:

Marco resin 28 C	100 g.
Monomer C	10 g.
Catalyst H C H	6 g.
Aecelerator E	6 ml.

The whole surface of the east is painted with the resin mixture, which is applied with a soft brush. As the working life is about eleven minutes at 20°C, the mixture must be applied immediately the accelerator has been incorporated, to allow the maximum time for it to wet the surface of the cast before it gels. It is necessary to brush the surface with the mixture several times to ensure that an even and complete film of resin is spread over the cast. Then the east is placed for three hours in a receptacle through which a draught of carbon dioxide is flowing (see p. 124). During this period the cast is supported by pressing the stalk into a lump of Plasticine. After three hours a clear glossy surface is produced, though maximum hardness is not developed for several days. Finally the resin stalk is cut off flush with the external auditory meatus. This is most easily done with a dental drill.

The cast can either be mounted on a wire cemented into the coloured resin in the external auditory meatus, or fixed in a rectangular Perspex container, which is filled with 50 per cent aqueous solution of glycerine, to which 5 per cent formalin is added to render it sterile. The latter method permits a much better view of the coloured cast of the cavities, by reducing the reflection and refraction caused by the curved surface of the transparent resin. It is desirable that some of the casts prepared by this method be mounted dry, and some in glycerine. When a cast is mounted in glycerine, one end of a piece of $\frac{3}{16}$ inch diameter Perspex rod is fixed with resin cement into a hole drilled into the coloured resin in the external auditory meatus. The other end of

the rod is cemented with Tensol No. 6 cement into a $\frac{3}{16}$ inch diameter hole drilled in one of the sides of the Perspex container.

The method of constructing Perspex containers is described in Chapter 8, and the method of fixing on the lid in Chapter 9. When the lid has been stuck on, the container is placed, almost completely full of the mounting fluid, in a vacuum chamber, and subjected to a water vacuum for at least an hour, to deaerate the fluid, and so ensure that no bubbles subsequently appear in it. Then the container is topped up until completely full and sealed by the method described on page 60. Figure 74 shows a medial view of a cast mounted in glycerine.

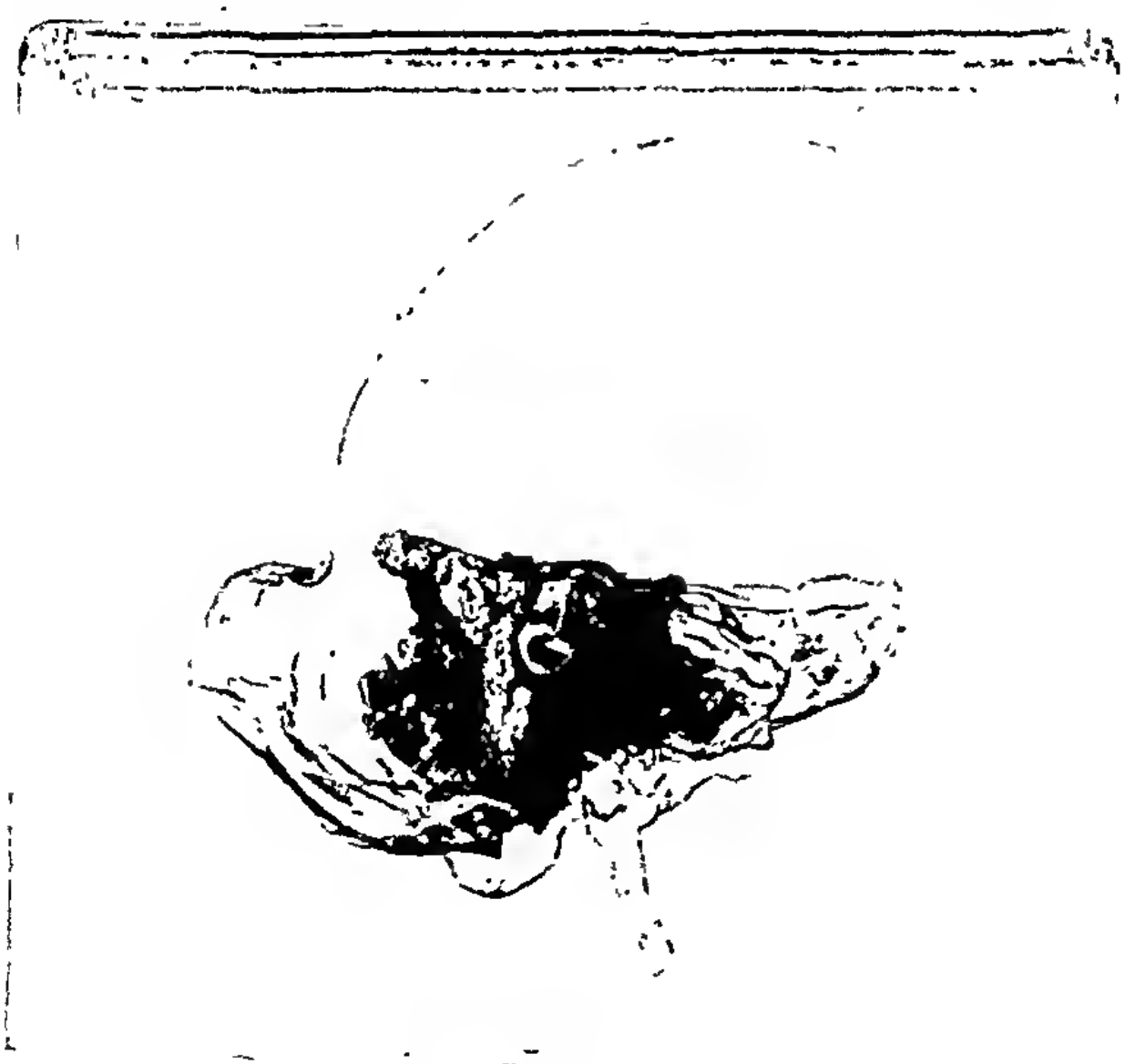


FIG. 74

Photograph of a coloured cast of the cavities of a left temporal bone, embedded in a transparent cast of the original bone. The resin cast is mounted in glycerine, to reduce the reflections and distortion caused by the curved surface of the transparent cast (*natural size*)

Chapter 23

CASTS FROM OTHER ORGANS

1. INTRODUCTION

PROVIDED that the worker is familiar with the properties of the resin and accessories, and with the general principles governing the use of the resin for anatomical casting, described in Chapters 16 and 17, and provided he has had adequate practical experience of this type of work, he should be able to prepare satisfactory corrosion casts from any part of the body, except those which require very specialised treatment, and which have been dealt with in previous chapters. But for the benefit of those who have little previous practical experience, and who wish to prepare casts from the liver, kidney or spleen, the details of the methods used for the preparation of casts from these organs are given in this chapter.

2. THE LIVER

An unfixed liver, removed at post mortem with as much as possible of the bile duct, the hepatic artery and the portal vein, is required for this technique. If the hepatic veins are also to be filled, the liver must be removed with at least half an inch of the vena cava projecting on either side.

Suitable Portex cannulae are tied into all the vessels and the bile duct. In the case of the vena cava, a cannula is tied to both the cut ends. Lengths of rubber tubing are fixed to each of the cannulae and adjustable screw clamps attached, so that the openings can be closed when required. If the vena cava has been cut rather close to the liver, it is necessary to fit cannulae made of flanged glass tube, instead of Portex tube, to ensure that the cannulae do not slip out.

The liver is immersed in cold water, and manipulated to remove any air which has entered the vessels. Then all ducts and vessels which are later to be filled with resin are washed out with deaerated water. An enema syringe is used to inject water into the bile duct and the hepatic artery, as considerable injection pressure must be applied, but water can be run into the

veins by gravity flow. The gall bladder is flushed out several times, by filling it with water and then, after the injection apparatus has been disconnected from the bile duct, compressing the bladder to expel the water.

The success of the resin injection depends largely on the thoroughness with which the ducts and vessels are washed out, but it is equally important that no air is introduced. The liver is fixed by injecting 5 per cent formalin into the hepatic artery, and placing it in a tank of 5 per cent formalin for forty-eight hours. It is not advisable to leave it in formalin for much longer than this, as it may become excessively hard.

Before the resin is injected, the ducts and vessels are again washed out with deaerated water. The Standard injection apparatus (see Fig. 29, p. 115) is used to fill all four systems with resin. As the bile ducts are very slender, it is advisable to fill them with a brightly coloured resin mixture, to make them as conspicuous as possible. Rather less viscous mixtures are required to fill the bile ducts and hepatic arteries, than that used to fill the portal and hepatic veins.

The following resin mixtures are recommended :

Bile duct.—3 units of the following mixture :

Marco resin 26 C	100 g.
Monomer C	25 g.
Crystic yellow powdered pigment M. 17	1 g.
or	
Crystic yellow pigment paste B. 258	2 g.
Catalyst H C H	4 g.
Accelerator E	4 ml.

Hepatic artery.—Three units of the same mixture as that used to fill the bile duct, but coloured with either red lake powdered pigment M. 11, or red pigment paste B. 214.

Portal vein.—Four units of the following mixture :

Marco resin 26 C	100 g.
Monomer C	15 g.
Red lake powdered pigment M. 11	0.5 g.

and

Blue powdered pigment M. 21	0.5 g.

or

Blue pigment paste B. 266	1 g.

and

Red pigment paste B. 214	1 g.
Catalyst H C H	4 g.
Accelerator E	4 ml.

These pigments give a purple colour. The exact proportions may be modified to produce the most effective contrast with the blue resin used to fill the hepatic veins.

Hepatic veins.—Five units of the same mixture as that used to fill the portal veins, but coloured with either blue powdered pigment M. 21 or blue pigment paste B. 266.

At a working temperature of 20°C, these mixtures have a working life of approximately fifteen minutes, but this is appreciably affected by the powdered pigments or pigment pastes. One unit of each mixture is used to make a test, by the method described on page 113, to determine the working life of each mixture.

The resin injection is made with the liver immersed in water at 30°C, and the liver is warmed up to this temperature, either by prolonged immersion in warm water, or by injecting warm water into the portal vein. Before the injection is commenced, the wall of the gall bladder is pierced with a fairly large round needle to make a puncture hole through which water, displaced by the resin, can escape.

Before the accelerator is added to the yellow pigment, about 25 ml. are decanted into a beaker and placed in the refrigerator for future use. The injection is commenced five minutes before the resin is due to gel. All the resin mixtures are allowed to flow in at the same time. The portal veins and hepatic veins are filled by gravity flow alone, from a height of about eighteen inches, the Standard apparatus being topped up when necessary to maintain this head of pressure. The injection of the bile duct and hepatic artery is assisted by the application of pressure by means of the enema syringe (see Fig. 29, p. 115, and text, p. 116), which is applied alternately for periods of half a minute to each tube.

In the case of the vena cava, before the injection of resin is commenced, the clamp attached to the rubber tubing which closes the end of the vena cava opposite to that from which the injection is being made is slightly opened to allow water in the vena cava, displaced by the resin to escape. As soon as resin begins to escape from this end of the vena cava, the clamp is closed.

In view of the considerable injection pressure used to fill both bile ducts and the hepatic artery, it is essential that all joints in the apparatus used are securely tied. If a joint becomes detached during the injection of the resin, there is no time to reconnect it, if the injection is being properly timed.

About half an hour after the resin has gelled, before it is really hard, the injection cannulae connecting the various ducts and vessels to the injection apparatus are cut through with a strong pair of scissors. More often than not, the cystic duct is too slender to allow enough resin to flow into the gall bladder to fill the latter completely, before the resin gels. The bladder is topped up by injecting some of the yellow resin placed in the refrigerator, by means of a hypodermic syringe, after the addition of accelerator. The needle is pushed through the wall of the gall bladder, and resin injected until it escapes through the escape hole previously made. The syringe must be emptied and cleaned before the resin in it gels, as it cannot be dissolved once it has set. An all-glass hypodermic syringe should be used in preference to a glass and metal one, as the acetone used to clean the syringe dissolves the cement with which the metal part is cemented to the glass barrel. The liver is left overnight in cold water, supported by cotton wool.

The next day, the ligatures tying the cannulae to the ducts and vessels are cut, and the remains of the cannulae removed from the resin casts which fill them. The ends of the casts of each of the ducts and vessels are sawn off to a convenient length. Part of the wall of the gall bladder is dissected away, and a resin rod is cemented with resin cement (see p. 104) to the casts of the gall bladder, the portal vein and the vena cava (see Fig. 75). A suitable rod can be made by filling a length of glass tube with resin, by the method described on page 193. The resin rod can be bent to the required shape by immersing it in boiling water until it is heated right through and then, after removal from the water, bending it, and holding it in the bent position until it cools and becomes rigid. It is essential to support the cast of the gall bladder, as the cast of the cystic duct is never adequate to support it. It is also necessary to support the cast of the hepatic veins, but the branches of the bile duct and hepatic arteries follow so closely the course of the portal vein, that the cast of the latter supports them adequately. Owing to the delicate nature of the

casts of the bile duct and the hepatic artery, it is not advisable to prepare casts of these without making at the same time a cast of the portal vein to support them.

The liver is left for eight days immersed in water while the resin hardens. The tissues are dissolved in hydrochloric acid. After forty-eight hours in acid, as much as possible of the macerated tissue is removed, by the method described on page 120. If maceration is not complete, the liver is returned to the acid for a further twenty-four hours. The macerated tissues are washed away, and the cast is pruned, cleaned, sprayed with a Marco resin 28 C mixture, and mounted, by the method described in Chapter 17. When a cast of the hepatic veins is included in the preparation, fully macerated tissues cannot be removed by washing alone. They have to be dissected away. Figure 75 shows a photograph of a cast of the bile duct, the hepatic artery, the portal vein and the hepatic veins. It is mounted with the gall bladder uppermost, as this view is most familiar to surgeons. Compare with Figure 21, page 68.

3. THE KIDNEY

Portex polythene cannulae are tied into the renal artery and the cut end of the ureter. The lumen of the latter is slightly stretched by inserting the points of iris dissecting forceps and gently opening the ends, so that a relatively large cannula can be inserted, as this facilitates the injection of the resin. Both the pelvis and the artery are washed out with deaerated water, and then the kidney is fixed for forty-eight hours in 5 per cent formalin. Before the resin is injected the pelvis and artery are again washed out with deaerated water.

The general procedure for filling the cavities and arteries of the kidney is the same as for the liver. Three units of red resin, and three of yellow are made by adding the appropriate colours to the following mixture :

Marco resin 26 C	100 g.
Monomer C	20 g.
Catalyst H C H	4 g.
Accelerator E	4 ml.

One unit of each resin mixture is used to make a test, by the method described on page 113, to determine the working life of the mixtures.

The resin is injected by means of the Standard injection apparatus (see Fig. 29, p. 115). The injection is commenced five minutes before the resin

is expected to gel. The injection tubes should be filled up so that there is an 18 inch head of pressure, and the enema syringe is only used to assist the flow of the resin if absolutely necessary. Great care is needed if the enema syringe is used to increase the rate of flow of resin into the ureter, as

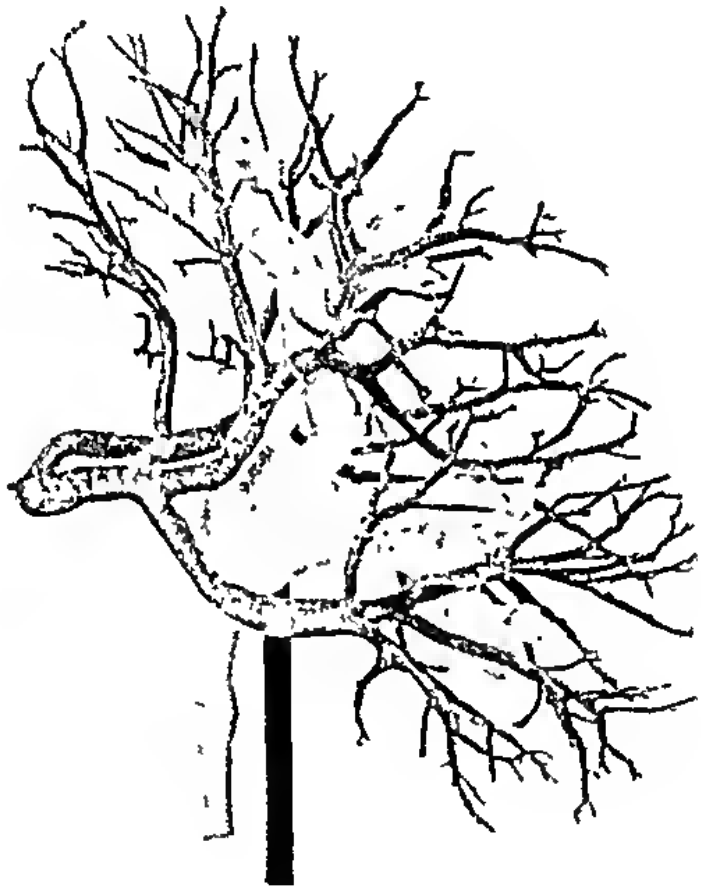


FIG 76

Photograph of a cast of the artery and cavities of a left kidney (natural size).

the pelvis is easily ruptured. Resin may also be forced from the extremities of the calices into the renal veins. The wall of the pelvis is pricked during the injection to provide an escape hole for any water which might otherwise be trapped in it. If the pelvis is not completely filled by the initial injection, its cavity is topped up in the same way as the gall bladder (see p. 217).

As quite small quantities of resin are required to fill the cavities and

artery, the success of the injection cannot be accurately gauged by the quantity of resin injected. Successful injection of the artery is indicated by the appearance of red resin in vessels on the surface of the kidney. When the pelvis is filled it becomes turgid, and coloured resin escapes from the prick in its wall.

The tissue of the kidney is destroyed by immersion in concentrated hydrochloric acid for twenty-four hours. Relatively little pruning is required, but if resin has escaped from the calices into the veins, bone forceps may have to be used to remove it.

The cast is pruned and cleaned. Then the cast of the arteries is cemented to the cast of the cavities. For although the cast of the arteries is held roughly in position around the cast of the cavities, there is a considerable amount of freedom of movement between the two casts. Finally the cast is sprayed with a Marco resin 28 C mixture, and mounted, by the method described in Chapter 17. Figure 76 shows a photograph of a cast of the renal artery and cavities of a kidney.

4. THE SPLEEN

The structure of the spleen is such that it is impossible to wash all the blood out of the organ. Consequently a procedure somewhat different from that usually followed is necessary, when casts of the arteries and veins of this organ are made.

The spleen should be obtained in as fresh a condition as possible. The arteries and veins are flushed out with a relatively small amount of deaerated water, which makes the organ quite turgid, as water does not readily diffuse through the capsule, or flow from the arteries to the veins. It is best not to fix the spleen, as this may result in coagulated blood diffusing into the vessels and obstructing the flow of resin.

After the vessels have been washed out, the spleen is left in water at 30°C for two hours, to allow sufficient time for it to be warmed right through, and for sufficient water to diffuse out to make room for the resin. The resin is injected by the Standard injection apparatus (see Fig. 29, p. 115), with an 18 inch head of pressure. The injection may be slightly assisted by the enema syringe, but excessive pressure must be avoided, as this causes extensive impregnation of the pulp with resin, and necessitates dissection of the macerated tissue to remove it from the cast of the vessels. The same resin mixture as that used to fill the cavities and arteries of the kidney, appropriately coloured, is suitable for injecting into the spleen.

PART IV

THE DIFFERENTIAL STAINING AND MOUNTING OF HUMAN BRAIN SLICES

Chapter 24

THE PREPARATION OF THE STAINED SLICES

1. INTRODUCTION

SINCE the publication in 1931 of Mulligan's technique for inhibiting the staining of the white matter in brain slices while the grey matter is being stained, by soaking them beforehand in a hot phenol solution, this procedure has formed the basis of all techniques for differential staining of the grey matter.

Mulligan used ferric tannate to stain the grey matter, but other workers have found that the results obtained with this stain are not consistent. Kampeier and Hospodar (1951) found that, of over 100 stains tested, by far the most consistent results were obtained with Berlin blue.

Although a number of papers have been published on this subject, none of the techniques recommended are entirely satisfactory. The method described below is not original. But as a result of modifications of the details of procedure recommended by various writers for the fixation of the brain, and for cutting, staining and mounting of the slices, consistently first-class results can be obtained.

2. MATERIALS AND METHOD

Brains removed at post mortem are fixed for two weeks in 10 per cent formalin, by the method described on page 64. Then the brain is washed in tap water, and the blood vessels which lie in the sulci are removed as completely as possible without serious damage to the brain, by tearing them away with dissecting forceps. Next, with the brain submerged in cold water, a strong jet of cold tap water is directed into all the grooves and fissures to wash out as much as possible of the blood pigment remaining in the broken ends of the blood vessels. This step is important, as the pigment not only discolours the adjacent grey matter, but also inhibits the subsequent staining of the grey matter with Berlin blue. After this treatment the brain is placed in clean 10 per cent formalin for a further four weeks.

Before the brain is sliced, gelatine solution is run into the great cerebral

fissure, between the hemispheres and the cerebellum, and into all sulci, allowed to set, and hardened by replacing the brain in formalin for a week. This treatment prevents fragmentation of some of the slices when the brain is sliced.

A gelatine solution suitable for this work is prepared by dissolving 20 g. powdered gelatine in 100 ml. water. It is applied in the following way. The brain is placed overnight in cold running water to remove formalin from its surface. It is then removed, and water clinging to its surface shaken off. Gelatine solution, only about two degrees Centigrade above its setting temperature of approximately 28°C, is run by means of a pipette to which a rubber teat is attached, into the fissures and sulci of one side. If the gelatine is used at the correct temperature, and provided that this work is not attempted when the room temperature is more than 22°C, the gelatine sets in the fissures and on the surface of the brain. When one side of the brain has been treated in this way, a jet of steam, provided by the apparatus shown in Figure 12 (p. 46), is directed on the gelatined surface of the brain just long enough to melt the gelatine on the surface, so that it flows down into the crevices. When the gelatine applied to one side has set, the next side is treated in the same way.

Two simple pieces of apparatus which can be easily constructed in the laboratory are recommended for slicing the brain. The first is shown in Figure 77 and consists of a Perspex base to which two arch-shaped Perspex knife-guides have been cemented. The guides are fixed just far enough apart to allow the knife used to slice the brain to slide easily down between them. A piece of cork sheet is placed on the Perspex base to protect the blade of the knife from damage.

This apparatus is used only to bisect the brain in whatever plane *e.g.* coronal, sagittal or transverse, it is intended to cut the slices. Two people are needed to cut the brain in half. One holds it in exactly the *desired position*, while the other slices it through with a 15 inch ham knife which has been very carefully sharpened on a hone until it is nearly as sharp as a razor. The knife blade must be entirely free from grease or oil, as this impairs the subsequent staining of the slices, and after each slice has been cut, the edge of the knife is carefully wiped to remove particles of connective tissue which get wrapped around it and which, if not removed, would scratch the surface of the next slice. The knife must be drawn through the brain with a continuous movement, to obtain slices with smooth surfaces.

Figure 78 shows the apparatus with which the slices are cut. It consists of a Perspex base surrounded by Perspex of the same thickness as the thickness of the slices. The triangular piece of Perspex guides the knife handle which

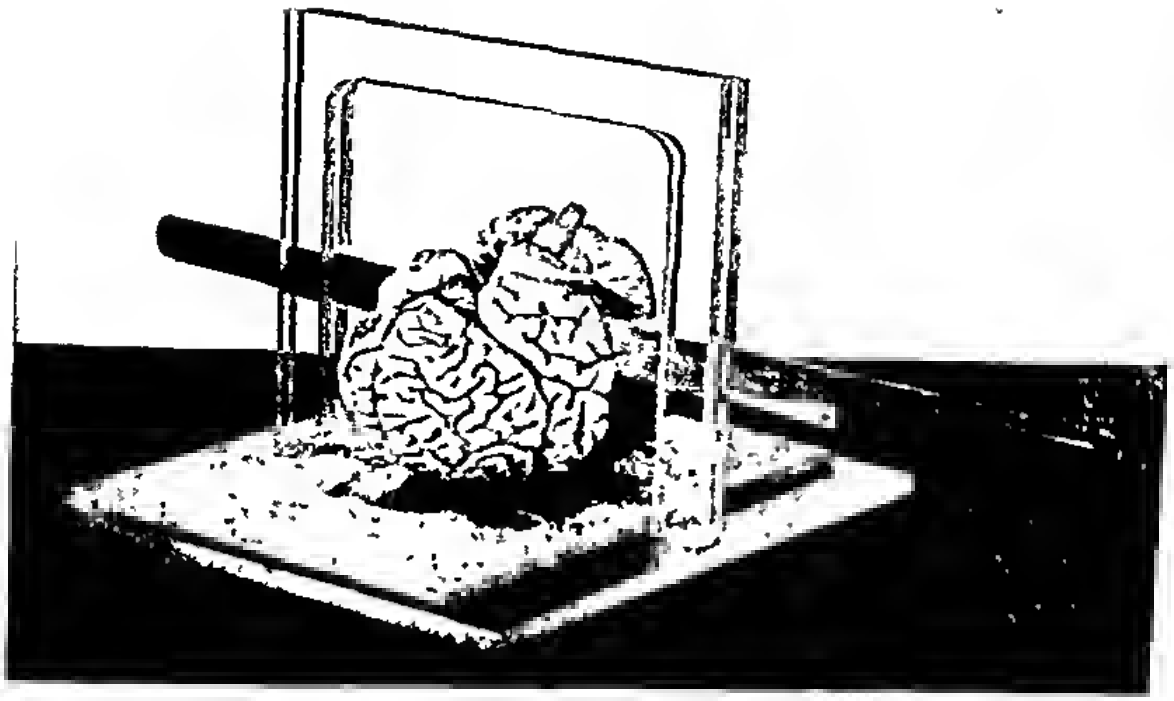


FIG 77

Simple apparatus constructed of Perspex for bisecting a brain in any desired plane When the apparatus is used, one person holds the brain steady, while the other uses the knife. The brain rests on a sheet of cork, to protect the knife blade from damage

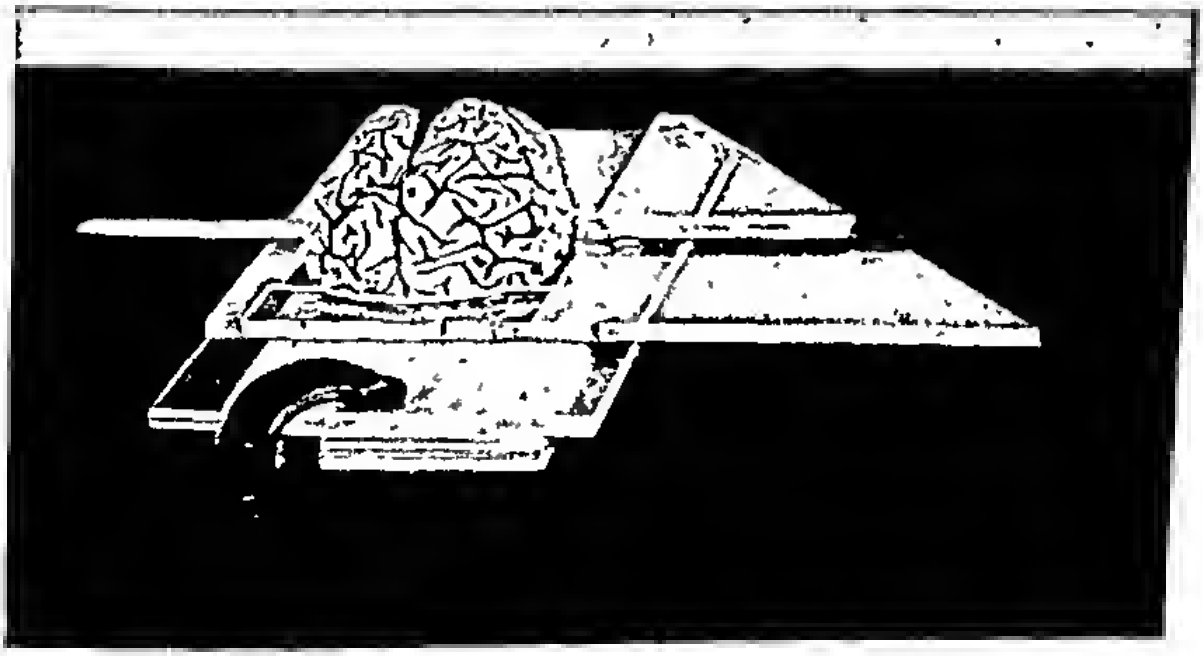


FIG 78

Simple apparatus constructed of Perspex for slicing a brain into relatively thick slices When the apparatus is used, one person holds the brain firmly against the Perspex base, while the other uses the knife The handle of the knife is so shaped that it slides along the edge of a triangular piece of Perspex.

is modified to fit against it securely, so that the blade is drawn across the brain as well as through it. If it is desired to cut slices thinner than those for which this apparatus was designed, a sheet of Perspex of appropriate thickness is placed in position so that the brain is raised up from the base of the apparatus. When this apparatus is used, it is first clamped securely to the bench. One person holds the brain firmly down on the Perspex, and against a curved piece of Perspex which prevents the brain from sliding, while the other uses the knife. Some practice is necessary to get really *good* slices, owing to a tendency for the brain to rise up from the Perspex base while a slice is being cut. This is due chiefly to lack of confidence on the part of the person holding the brain down in the person wielding the knife. It is recommended that the slices should normally be 1 cm. thick, as this is a convenient thickness for easy handling. However, no special difficulty is encountered if the slices are cut 0.5 cm. thick.

Immediately after the slices have been cut they are washed in running water for an hour to remove blood oozing out of the cut ends of blood vessels. Remains of blood vessels are carefully pulled away from each slice while it is immersed in water. The slices are then placed in 10 per cent formalin for at least forty-eight hours before the staining is done. If the brain is in a satisfactory condition, which will permit first-class differential staining, the grey matter of the slices is a very pale pinkish brown colour. If it is a rusty yellow, it has been stained by iron salts from the blood and does not take the stain well.

During the staining process the slices are handled with suitably shaped lifters made of Perspex. As the stain is a surface one, the slices must be handled gently but, to ensure that both surfaces are equally stained, they must be moved about in the fluids during the staining process, to ensure equal access of the reagents to both sides. Pyrex pie dishes are suitable for holding the various fluids, and 1 litre of each reagent is sufficient to stain a whole brain cut into slices 1 cm. thick.

The slices are stained in the following way :

1. Slices are washed in running cold water for one hour.

2. Each slice is immersed for *five minutes* in a solution made up by dissolving 50 g. phenol crystals and 5 g. copper sulphate crystals in 1000 ml. distilled water, and adding 1.25 ml. concentrated hydrochloric acid. This solution is maintained at 60°C. by placing the dish on a hot plate. The dish is covered with a piece of glass to reduce evaporation of its contents.

3. The slice is immersed in iced water for ten seconds. If the slice is washed for longer than this, subsequent differentiation is less striking.

4. The slice is immersed in freshly prepared 2 per cent ferric chloride solution, made up in distilled water, for a period of between forty-five seconds and one minute, according to the intensity of the staining required. If the slice is stained for longer in this solution, the white matter begins to absorb the stain as well as the grey. As the grey matter absorbs the ferric chloride, it turns light brown.

5. The slice is washed in gently running cold water for one minute. More prolonged washing or washing in rapidly flowing water removes so much of the ferric chloride solution from the grey matter, that in the next stage of the staining a pale blue instead of a dark colour is produced.

6. The slice is immersed in freshly prepared 1 per cent potassium ferrocyanide solution, made up in distilled water, for four minutes. This converts the ferric chloride retained by the grey matter into ferric ferrocyanide or Berlin blue. More prolonged immersion in this solution does not produce a darker blue, as in four minutes all the ferric chloride is converted into Berlin blue. However, the blue colour is at first comparatively milky in tone, and continues to darken for some time after the slice has been removed from the potassium ferrocyanide solution. Therefore if a series of slices are being stained, and all are required to be stained to the same degree, the time during which the slices remain in the ferric chloride must not be increased just because it is observed that slices stained very recently are a lighter shade of blue than those stained half an hour previously. Consistent results are obtained by treating each slice in exactly the same way.

7. The slice is placed overnight in running cold water. Each slice is then supported on a sheet of Perspex held under water, while each side in turn is washed with a fairly strong jet of cold water from the tap. This treatment removes a slimy film, originally produced by the hot phenol solution, from the surface of the white matter.

The slices are stored for at least a month in about five litres of a solution prepared by mixing 75 ml. distilled water, 25 ml. pure glycerine, 10 ml. formalin and 0.2 g. citric acid crystals. The slices must be protected from bright light, as this causes the blue stain to fade. The solution must be slightly acid to prevent hydrolysis of the stain, which causes it to turn green. Fading is an irreversible reaction, but hydrolysis can be reversed by placing the slices in a weak acid solution. The slices should be left in the solution recommended above for at least a month before they are mounted, as they

turn the fluid slightly milky. Provided that they are adequately protected from light, they can be stored in this solution indefinitely. But care must be taken not to add more than the recommended amount of citric acid, as otherwise the white matter absorbs the acid sufficiently to be stained

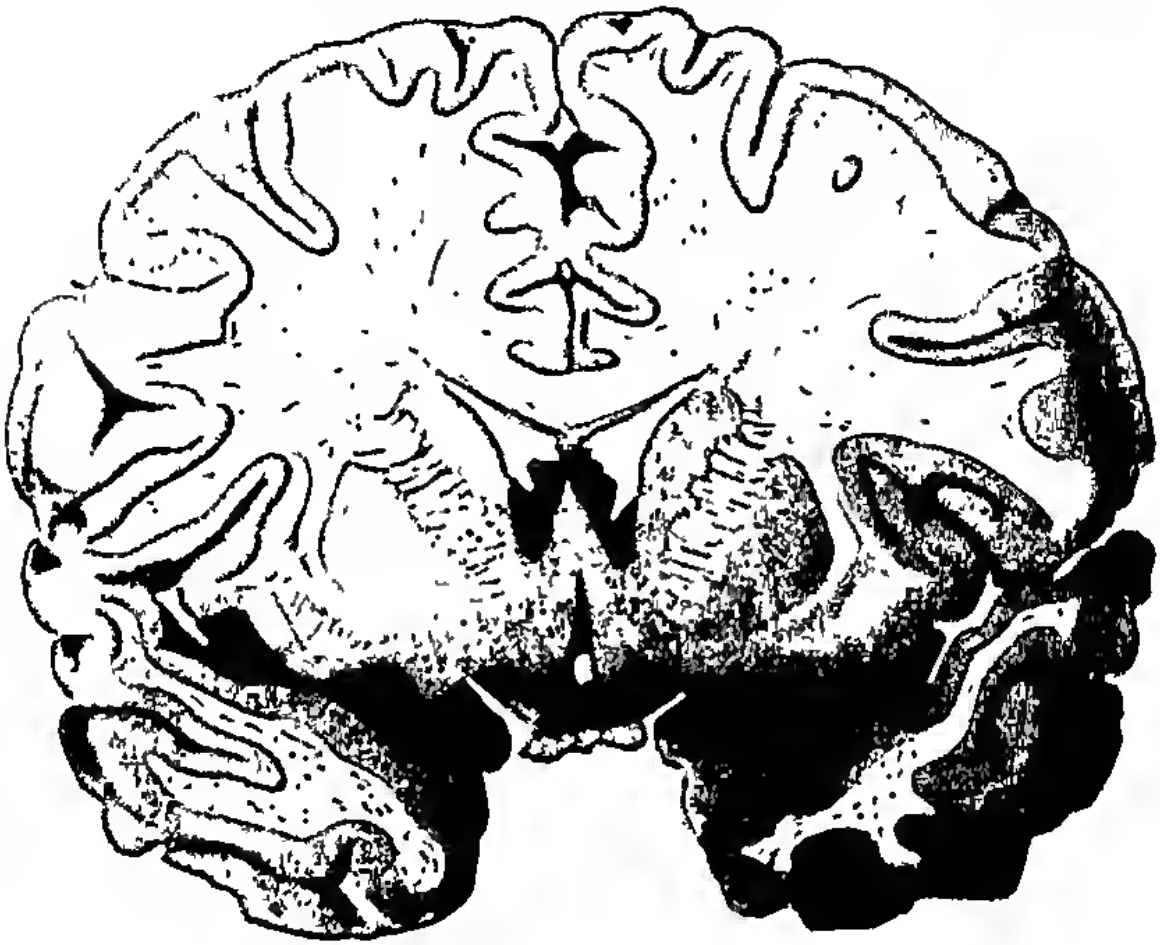


FIG. 79

Photograph of a differentially stained brain slice (natural size).

yellow. Although the gelatine which holds the various parts of individual slices together is also stained dark blue, this does not impair the value of the specimens, or seriously spoil their aesthetic appearance. But on no account must the mounting solution be acidified with hydrochloric acid instead of the citric acid recommended, as hydrochloric acid, even in very dilute solution, destroys the gelatine. Figure 79 shows a typical brain slice stained by the method described above.

REFERENCES

KAUFMANN, O. F., & HOSFORD, E. W. (1951). Mounting of stained serial slices of the brain as wet specimens in transparent plastic. *Anat. Rec.* 110, 1-15

MULLIGAN, J. H. (1931). A method of staining the brain for macroscopic study. *J. Anat., Lond.* 65, 465-472

Chapter 25

MOUNTING AND DISPLAY

EACH slice is sewn to a rectangular sheet of 1/16 inch Perspex by means of No. 0. Chinese twist. A small circular piece of celluloid, punched out of an old film, is incorporated in each stitch on the side of the slice furthest from the Perspex sheet, to prevent the silk twist from cutting into the brain. About eight stitches are required to fix a coronal slice securely. The stitches must be tight enough to prevent the slice from rubbing against the sheet of Perspex to which it is sewn, as vibration would in time result in the blue surface stain being rubbed off. The tension of each stitch should be such that the celluloid disc just begins to sink below the general level of the surface of the slice.

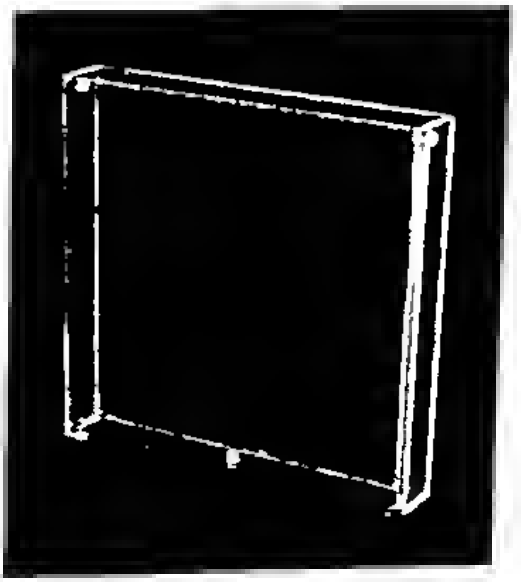


FIG. 80

Photograph of one of the Perspex containers in which differentially stained brain slices are mounted.

The sheets of Perspex to which the slices are fixed are mounted in fluid in rectangular boxes. Figure 80 shows one of the boxes, and Figure 81 indicates the details of the actual mounting. It is essential that the width of the box is such that the surface of the slice cannot rub against the Perspex. The method of constructing Perspex containers is given in Chapter 8.

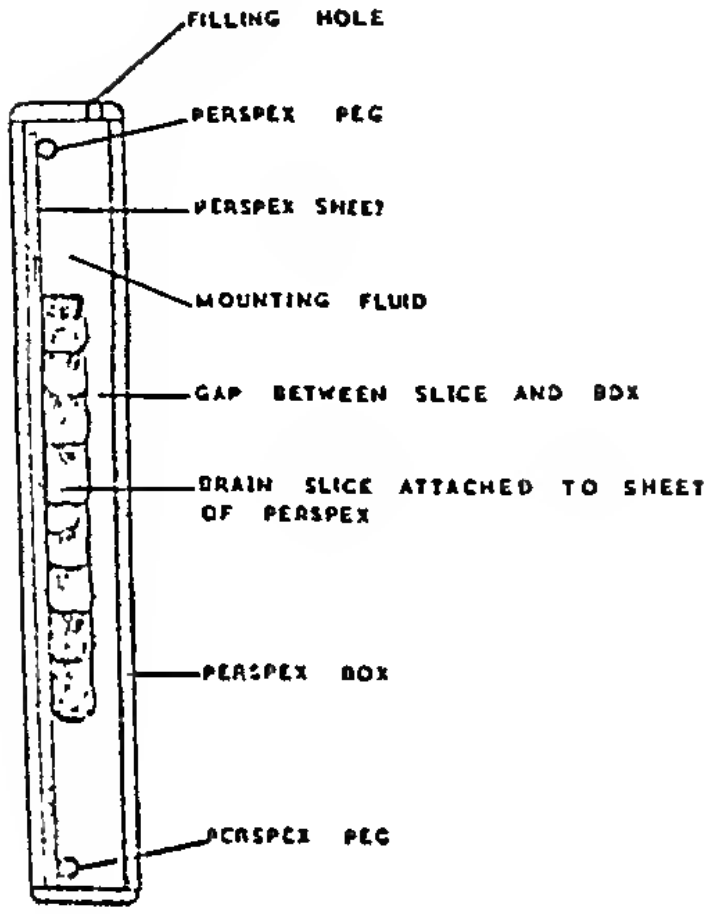


FIG. 81

Diagram to show the details of mounting a brain slice in a Perspex container. The stitches by which the slice is fixed to the Perspex sheet are not shown.

The method of sticking on the lid and sealing of Perspex containers is given in Chapter 9. In the case of containers for brain slices two modifications are necessary. The sides of the container must be covered with black paper, and black paper stuck over the lid, so that the mercury arc lamp used to polymerise the cement can only fall on the actual joint, as otherwise some fading of the blue stain occurs. When the lid has been cemented on, the mounting fluid is emptied out of the container, which is then filled exactly 9/10 full of a solution made by mixing 75 ml. distilled water with 25 ml. pure glycerine. The container is then placed in a vacuum chamber (the details of a suitably shaped vacuum chamber constructed of Perspex are shown in Figure 82), and evacuated. The vacuum chamber is rocked and tilted to dislodge air trapped around the slice, and then the pressure is restored to normal. The vacuum treatment is repeated once. Any small air bubbles

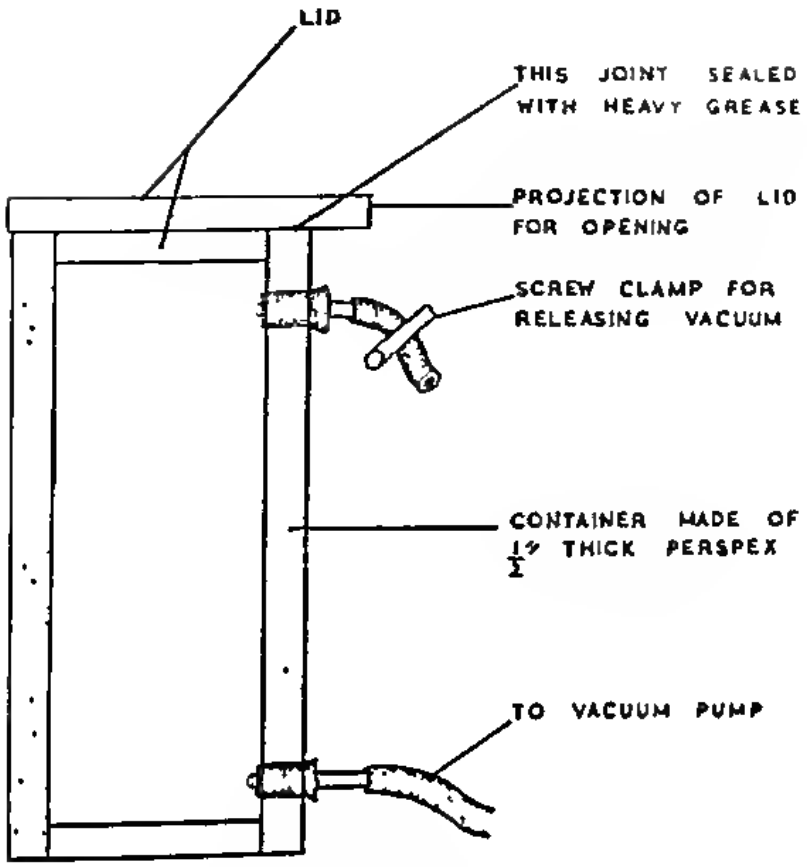


FIG. 82

Diagram of a vacuum chamber made of Perspex, to accommodate containers used for mounting brain slices. Note the Perspex stuck to the under surface of the lid to prevent the walls of the chamber collapsing under vacuum.

remaining soon disappear, the air being dissolved in the deaerated mounting fluid.

The box is next topped with filtered formalin containing 2 per cent citric acid, until it is completely full. It is sealed by the method described on page 61. This procedure results in the mounting fluid being completely devoid of air bubbles. If subsequently air bubbles appear, this indicates that one of the joints of the container is defective, and the only satisfactory remedy is to remount the slice in a new container.

As the blue stain fades in time when exposed to light, it is advisable to store the specimens in a light-proof box when not in use. A convenient method of storage is provided by a wooden box into which a set of mounted brain slices fit in ventrical slots, as shown in Figure 83. The slots should be lined with velveteen to avoid scratching the Perspex containers, and it is also advisable to line the floor of the box with foam rubber sheeting, to reduce

FIG. 83

Wooden storage box for a set of coronal brain slices. The slots are lined with velveteen to prevent the Perspex being scratched, and the floor is lined with foam rubber sheeting to reduce jolts when the specimens are replaced.

to a minimum the shock when specimens are replaced. If stored under normal conditions, the slices remain serviceable for at least ten years. When it is necessary to replace them the same Perspex containers and storage box can be used again.

Appendix

Proprietary materials referred to in the text, with addresses of manufacturers.

Accelerator E	*see under* Marco Resin.
Allen & Hanburys Ltd.	48 Wigmore Street, London, W.1.
Aluminium wire	P. Ormiston & Sons Ltd., 31a Denmark Road, London, W.13.
Arc Lamps	*see under* Mercury.
Aural syringe and Allen's metal pipe (for applying Tensol Cement No. 3)	Down Bros. & Mayer & Phelps, 32 New Cavendish Street, London, W.1.
Barrier Cream	*see under* Innova.
Bostic adhesive No. 252	B.B. Chemical Co. Ltd., Leicester, England.
Boston Neoprene latex and colour dispersions	B.B. Chemical Co. Ltd., Leicester, England.
Brushing Belco	*see under* Naylor's.
Carbon Pencils	E. Wolff & Son, Britannia Pencil Works, Neasdon, London, N.W.10.
Catalyst H C H	*see under* Marco Resin.
Chinagraph pencils (for writing on glass and Perspex)	E. Wolff & Son, Britannia Pencil Works, Neasdon, London, N.W.10.
Collapsible lead tubes (available in boxes of 36 or more)	Flexile Metal Co. Ltd., 796 Holloway Road, London, N.19.
Colour dispersions	*see under* Boston.
Crystic pigment pastes and powdered pigments	*see under* Marco Resin.
Crystic resin 182	*see under* Marco Resin.
Dentruset modelling plaster	Dental Manufacturing Co. Ltd., Brook House, 97 Great Portland Street, London, W.1.
Homacol liquid toilet soap	Horton Manufacturing Co., Rickmansworth, Herts., England.

Polyvinyl alcohol	*see under* Mowiol
Portex polythene tubing (with bore from 0.5 mm. to 7.5 mm.) and Portex vinyl VY standard tubing (for larger sizes)	Portland Plastics Ltd., Bassett House, Hythe, Kent.
Sable water-colour brushes . . (Series 7)	Windsor & Newton Ltd., Rathbone Place, London, W.1.
Speera binocular magnifying spectacles	W. Watson & Sons Ltd., 313 *High Holborn*, London, W.C.1.
Syringe	*see under* Aural.
Tensol cement	*see under* Perspex.
Wax (modelling) . . .	*see under* Number 4.
Whatman's hotpressed water-colour drawing board	Windsor & Newton Ltd Rathbone Place, London, W.1.

Polyvinyl alcohol	*see under* Mowiol
Portex polythene tubing (with bore from 0.5 mm. to 7.5 mm.) and Portex vinyl VY standard tubing (for larger sizes)	Portland Plastics Ltd, Bristol Road, Hythe, Kent.
Sable water-colour brushes . . (Series 7)	Windsor & Newton Ltd, Rathbone Place, London, W.1.
Speera binocular magnifying spectacles	W. Watson & Sons Ltd, High Holborn, London, W.C.1.
Syringe	*see under* Aural.
Tensol cement	*see under* Perspex.
Wax (modelling) . . .	*see under* Number 4
Whatman's hotpressed water-colour drawing board	Windsor & Newton Ltd, Rathbone Place, London, W.1.

Innoxa Barrier cream B.W.2. . . (marketed overseas under the trade name Kerodex [in France Isolex]).	Scientific Pharmacals Ltd., 1 Eden Street, London, N.W.1.
Kaffir D Plaster	Cafferata & Co., Newark, Notts., England.
Magnifying spectacles . . .	*see under* Speera.
Manoxol O.T. 20% solution . .	Hardman & Holden Ltd., Manox House, Canal Street, Miles Platting, Manchester, 10, England.
Mandarin waterproof black carbon drawing ink	Windsor & Newton Ltd., Rathbone Place, London, W.1.
Marco Resin, 26 C & 28 C . . Accelerator E Catalyst H C H Crystic pigment pastes and powdered pigments. Crystic resin 182 Monomer C	Scott Bader & Co. Ltd., 109 Kingsway, London, W.C.2.
Mercury arc lamps GES MA/H clear 400 watt and GES lamp holders and chokes. (for polymerising Tensol cement No. 3.)	General Electric Co. Ltd., Kingsway, London, W.C.2.
Monomer C	*see under* Marco Resin.
Mowiol 50-88	Lawfer Chemical Co. Ltd., 27 Regent Street, London, S.W.1.
Naylor's Brushing Belco (available in ¼ pint tins)	Imperial Chemical Industries Ltd., Paints Division, London, W.1.
Number 4 toughened wax . .	Dental Manufacturing Co. Ltd., Brock House, 97 Great Portland Street, London, W.1.
Paragon scalpels, handles and blades	Paragon Razor Co., Sheffield, England.
Perspex . . . . . Perspex No. 3 (antistatic) polish Tensol cement No. 3. Tensol cement No. 6.	Imperial Chemical Industries Ltd., Plastics Division, Welwyn Garden City, Herts, England.
Philips blended lamps . . .	Philips Electrical Ltd., Century House, Shaftesbury Avenue, London, W.C.2.
Plaster	*see under* Dentruset and Kaffir D.
Plasticine	Harbutt's Plasticine Works & Studio, Bathampton, Bath, England.
Polish	*see under* Perspex.

Polyvinyl alcohol	*see under* Mowiol
Portex polythene tubing (with bore from 0.5 mm. to 7.5 mm.) and Portex vinyl VY standard tubing (for larger sizes)	Portland Plastics Ltd., Bassett Road, Hythe, Kent.
Sable water-colour brushes . . (Series 7)	Windsor & Newton Ltd., 6 Rathbone Place, London, W.1.
Speera binocular magnifying spectacles	W. Watson & Sons Ltd., 313 High Holborn, London, W.C.1.
Syringe	*see under* Aural.
Tensol cement	*see under* Perspex.
Wax (modelling) . . .	*see under* Number 4
Whatman's hotpressed water-colour drawing board	Windsor & Newton Ltd., Rathbone Place, London, W.1.

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